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RHEOSPORANGIUM APHANIDERMATUS, A NEW GENUS AND SPECIES OF FUNGUS PARASITIC ON SUGAR BEETS AND RADISHES

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INTRODUCTION

The fungus to be described in this paper was originally secured from damped-off seedlings of sugar beets (*Beta vulgaris*) which were grown in soil that had previously produced the black-root of the radish (*Raphanus sativus*). It was at first mistaken for *Aphanomyces laevis* De Bary and was so considered in a preliminary note.[1] The organism stands in a causal relation to both of the diseases mentioned, and recent trials have shown that it retains its virulence after a continuance of 30 months in artificial culture. The pathogenic relations of the organism were discussed in a recent paper,[2] but the fungus was not named or treated in its taxonomic relations. The present paper deals with the results of such further studies involving the morphology, cytology, and taxonomy of the organism as were found necessary to establish its identity and relationships and to make clear the various stages of its life history.

LIFE HISTORY AND GROSS MORPHOLOGY OF THE ORGANISM

In the general character of the disease produced in seedlings and in its appearance in cultures the organism resembles *Pythium debaryanum* so closely as to be readily confused with it, except in the asexual fruiting stage. The vegetative mycelium consists of nonseptate hyphæ which develop a profuse white aerial growth on suitable solid media, such as string-bean or oatmeal agar and certain cooked vegetables, of which the string bean is one of the most satisfactory. Oospores are formed in

[1] Edson, H. A. Damping-off and root rot parasites of sugar beets. *In* Phytopathology, v. 3, no. 1, p. 76. 1913.

[2] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour. Agr. Research, v. 4, no. 2, p. 135–168, pl. 16–26. 1915.

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July 15, 1915
G—50

considerable numbers on string-bean agar. The normal life history, however, can be observed only under aquatic conditions. Cultures upon sugar-beet seedlings in water in Petri dishes where abundant aeration was obtained have been employed in the studies discussed in this paper.

A profuse, hyaline, nonseptate, branching mycelium with a finely granular internal structure develops (Pl. XLIV, fig. 12). The young hyphæ vary from 2.8 to 7.3μ in width, while portions of threads destined to function in reproduction may considerably exceed that diameter. Cultures 1 or 2 days old which have been well aerated at favorable (warm) temperatures exhibit remarkable protoplasmic streamings which, so far as they have been observed, are always directed toward the distal ends of the hyphæ. At such times the protoplasmic granules, or mitochondria, to which attention will be directed presently, may be seen to change their relative positions constantly and to exhibit a more or less independent motion. This protoplasmic streaming leads to an accumulation of material in the extremities of the threads, in consequence of which they become enlarged and more or less distorted according to the conditions. At length a partition wall is laid down to cut off the swollen portion from the rest of the mycelium; thus the first step in the process of fructification is accomplished (Pl. XLIV, fig. 7).

The body thus cut off merits special attention. It is similar in general appearance to the zoosporangia of various types which develop in the Saproligneaceae; but a study of its function shows that it differs distinctly from them, since it gives rise, not to zoospores, but to the body in which the zoospores are formed. While closely related in origin and appearance to the sporangium, it is correlated with important modifications in the process of zoogenesis which have not previously been described and constitutes a special organ with a new and sufficiently differentiated function to justify its designation by a distinctive name. The term "presporangium" is therefore applied to it. When fully formed, this body varies greatly in size and shape, depending upon the point where the cross wall is laid down. The presporangium may vary in length from less than 50 to more than 1,000μ. The width may scarcely exceed that of undifferentiated hyphæ, or it may increase to 20μ. Branching is present or absent according to the character of the segmented portion of the hypha before metamorphosis. After being cut off, these bodies increase in diameter, taking on a distinctly swollen and more or less distorted appearance. This step is accomplished apparently by taking up water which accumulates in vacuoles (Pl. XLIV, fig. 13, and Pl. XLVIII, fig. 1). The consequent development of turgor eventually becomes great enough to rupture the wall of the presporangium and permit the discharge of its contents, which emerge in an uncleaved condition (Pl. XLIV, fig. 6, 10). The rupture usually occurs at the extreme end of the hypha, though it sometimes takes place at the tip of one of

the side branches, which now present the appearance of swollen protuberances (Pl. XLIV, fig. 7).

The time at which the rupture of a given presporangium may be expected to occur can be predicted with reasonable accuracy some little time in advance by the appearance of the vacuoles, especially by one which develops at the tip so as to produce a relatively large hyaline area at the point where the break is to occur. This fact makes it easy to observe, with the oil- or water-immersion lens, the expulsion of the contents and its subsequent cleavage from the beginning, because in hanging-drop cultures the field within which it is to appear may be brought into focus before the phenomena begin to develop.

When the rupture occurs, there is at first a rush of protoplasm from the presporangium, soon to be modified to a steady flow of diminishing velocity. The entire protoplasmic contents flow out, leaving only the empty wall. The new body thus formed may be seen to be inclosed in a thin, membranous, almost invisible and very plastic wall, which is so flexible that the discharging mass takes on a spherical form as it is relieved from the pressure of the presporangium wall. During egress, therefore, the delivered portion presents the appearance of an enlarging sphere of protoplasm, while during the later part of the process the inclosed portion of the membranous wall may be seen advancing along the presporangium cavity, drawing out with it the last portions of the contents. This body, which is a zoosporangium, remains at the mouth of the presporangium wall during cleavage, although it does not appear to be attached to it by any visible means. Promptly following the egress of the sporangium, its cytoplasm cleaves into zoospores, which are liberated by the rupture of the sporangium wall.

The various steps in the process have been followed in the living material in hundreds of cases, as well as in the sections. In a typical instance observed in a hanging-drop culture under a water-immersion lens the liberation from the presporangium was completed at 11 a. m. Cleavage lines first appeared as indistinct grooves at 11.07. Short cilia were observed waving at the periphery at 11.09, and at 11.10 a rocking motion of the entire mass began. This continued uninterruptedly during the remaining time. The spores began to assume a definite outline at 11.13 and exhibited individual motion at 11.17. A large vacuole in each spore had become distinctly visible at 11.18. The spores began to change their relative positions quite freely at 11.25. They had assumed the normal adult shape at 11.26 and were swimming about with great activity within the sporangium. They escaped at 11.29 through a puncture produced in the membrane by the force of their impact upon it. In this instance 24 spores were counted. The number was commonly somewhat larger, approximating 50, although instances of as few as 4 were seen, and sporangia containing a number estimated to be 150 or

200 were quite frequent. In the majority of cases the enveloping membrane seemed to burst and then contract so as to allow the zoospores to escape almost simultaneously in all directions. It is usually impossible to discover any trace of the membrane after the spores have escaped. During the progress of cleavage the wall seems to function as a semipermeable membrane, since the distance between it and the cytoplasm increases somewhat and the size of the entire body increases by a few microns. The time which elapses between the egress from the presporangium and the liberation of the zoospores is usually about 30 minutes, though in some instances observed the entire process occupied only 17 minutes.

Under favorable conditions of observation at high magnification one may occasionally note an additional phenomenon, which appears to be related to the first appearance of cleavage furrows at the periphery of the contents of the sporangium. About the time the first indications of cilia are observable small, bubble-like protuberances suddenly puff out here and there and then instantly disappear as if they had burst. Thereafter cleavage indentations appear at the points of rupture, which continue to deepen until cleavage has been completed.

In a few instances the presporangium was observed to develop within the tissues of the host or in submerged agar in such a way as to permit of its reenforcement by the surrounding substance to such an extent that it was not ruptured, but remained intact. In such cases cleavage occurred within the presporangium, and the spores either escaped from the tip of the hypha for a swarm period or remained imprisoned within it, where they germinated. Instances were also observed in which germination occurred within the sporangium without liberation. Such cases, however, were seen only rarely and are not to be regarded as typical.

Following their escape, the zoospores swim about actively for a time, then come to rest, round up, increase in size to a diameter of about 11 or 12μ while developing a large central vacuole, and send out germ tubes, generally two, which develop into typical vegetative mycelium from which zoospores are again produced. In the motile stage the zoospores are plano-convex, with a rather deep sinus on the flattened side. They always possess a single somewhat conspicuous vacuole and are biciliate. They have an average length of 12μ and an average width of 7.5μ.

Coincident with the later stages of zoospore production and following it, oospores are formed. The oogonium develops terminally as a spherical body from 22 to 27μ in diameter. The antheridium, which develops either terminally or more generally in an intercalary position, is appressed to the oogonium. It is suborbicular, becoming cylindric to broadly clavate, averaging from 9 to 11μ in width by 10 to 14μ in length. The oospores are spherical and when mature have either a smooth or some-

what roughly undulated wall, averaging from 1.5 to 2.5μ in thickness. They have an average diameter of from 17 to 19μ (Pl. XLV, fig. 7).

Germination of the oospores was observed several times in the course of the studies. Water cultures in Petri dishes containing young sterilized sugar-beet seedlings were employed for growing the spores, which were produced literally by the thousand. After their development the cultures were allowed to dry out slowly without removing the covers from the dishes. When they had remained apparently air-dry for one month, sterile water was added to certain of them and they were observed for evidences of growth. In three out of five cultures zoospores were seen on the second day, but microscopic examination failed to demonstrate the presence of germinating oospores. While it hardly seems possible that the vegetative mycelium or asexual spores of the fungus would survive this drying on a glass plate for a month, there is reasonable ground for doubt as to the source of the growth. On June 15 similar cultures started in November and in January were tested for oospore germination in the same way. During the following three or four days, four out of five cultures yielded positive results which could be confirmed by microscopic examination. Of the many thousand spores present, however, only a very few germinated. These invariably put out germ tubes, which developed into vegetative mycelium bearing the characteristic asexual fruiting bodies.

When the available food supply has been consumed or the water has become sufficiently exhausted by gradual desiccation, aquatic cultures supplement by a further effort at preservation the fructifications already discussed. The cytoplasm which has not been used in spore formation collects in masses in different parts of the hyphæ, and walls itself off. These accumulations are most frequently found at the ends of threads, where they appear like small presporangia (Pl. XLV, fig. 6); but they occur also in other positions. They are more resistant to desiccation than ordinary hyphæ and doubtless serve under natural conditions to carry the organism through brief periods of drought.

DETAILED MORPHOLOGY OF THE ORGANISM

For the purpose of clearing up certain important details of the processes discussed in the preceding paragraphs, material from water cultures at various stages of the life history was killed in Flemming's weaker solution diluted with water, and embedded in paraffin, then sectioned and stained with Flemming's safranine, gentian violet, and orange G. For cilia the gentian alone was used. Camera-lucida drawings from this material are used to illustrate the following discussion.

The slides show that the nuclear divisions which precede oospore production occur only in the older portions of the hyphæ (Pl. XLIV, fig. 2, 3, 4, 5, 8, 9, 11). Following karyokinesis the daughter nuclei are carried

by the protoplasmic stream to the developing presporangium in which no divisions occur. The number of nuclei assembled before the organ is segmented off by a cross wall varies greatly. Presporangia containing as few as 4 were seen, while 200 or more were not uncommon. In the one illustrated 162 are shown (Pl. XLIV, fig. 7). The nucleus at this stage is spherical to oval and contains typically a single nucleolus located at one side, frequently protruding slightly from the body of the nucleus (Pl. XLVI, fig. 1, 8). In some cases, especially in the zoospore, the nucleus may contain two or even three nucleoli. The mitochondria, which are numerous in the vegetative hyphæ (Pl. XLVI, fig. 8), where, in certain stages at least, they are arranged so as to suggest a peripheral distribution, are still more abundant in the presporangia (Pl. XLVI, fig. 1), where they are evenly distributed throughout in great numbers. Following the development of a division wall to cut off the presporangium, numerous vacuoles develop within that body (Pl. XLVIII, fig. 1). These coalesce (Pl. XLIV, fig. 13) as they increase in size and develop the pressure which results in the rupture of the inclosing wall (Pl. XLIV, fig. 6, 10). Plate XLIV, figure 6, shows the condition just at the instant following rupture. The thin wall of the sporangium is seen covering the protruding cytoplasm, which is just beginning to escape. The sudden relief of tension is shown in a striking manner by the influence temporarily exerted on the form of the nuclei at the narrower portion of the body. Plate XLIV, figure 10, represents the process at a later stage. Here the rupture took place at the tip of one of the branches. At two places within the wall of the presporangium the membranous wall of the sporangium is seen receding along the cavity, while the flexibility of its structure may be judged from the spherical shape of the delivered portion.

Sections of the young zoosporangium show at first an entirely undifferentiated condition to be followed by the development of vacuoles and the migration of nuclei toward the periphery (Pl. XLV, fig. 5). The vacuoles coalesce, developing so as to form a relatively large irregular central cavity from which cleavage lines split outward. Meantime the nuclei arrange themselves at a uniform distance from the outer surface with their nucleoli turned toward the center of the mass (Pl. XLV, fig. 1). Cleavage furrows soon become apparent on the periphery so as to delineate the outlines of the future spores (Pl. XLV, fig. 4). Reference has already been made to the first appearance of these furrows on the exterior in living material. A single large vacuole develops near each of the nuclei (Pl. XLV, fig. 2, 4) and cilia appear in the indented areas between the future spores (Pl. XLIV, fig. 3). In this way a single row of zoospores is cut out at the periphery of the sporangium (Pl. XLV, fig. 2).

Cleavage usually progresses somewhat more rapidly in one side of the sporangium than in the other. It sometimes happens that the first

spores to mature succeed in rupturing the sporangium wall before the others have become mature. Such attached spores are often seen struggling about in the water until the completion of cleavage releases them from each other and they swim away. As already noted, cleavage may occur within the sporangium without its escape from the wall of the presporangium. In such cases the sporangium wall draws away from that of the presporangium so as to make it distinctly visible (Pl. XLIV, fig. 1).

The mature zoospores are uninucleate; hence, the number to develop in a given sporangium is predetermined by the number of nuclei contained within the presporangium. The number varies within wide limits; but, so far as accurate counts have been made, it has always been even. The zoospore nucleus is distinctly top-shaped, with the nucleolus at the broad end. The pointed end is directed toward the flattened side of the spore, where it ends in a blepharoplast, from which the cilia arise (Pl. XLV, fig. 3, and XLVI, fig. 6, 7, 9, 10, 11). The spores are plano-convex or slightly concavo-convex, with a sinus in the flattened side, which in the uncleaved sporangium is directed outward. The blepharoplast appears to come in contact with the spore membrane at the base of this sinus (Pl. XLVI, fig. 9, 10, 11).

It has already been pointed out that the cilia become visible in the living material before cleavage is complete. The sectioned material bears out this observation (Pl. XLV, fig. 3). When first observed in hanging-drop preparations, the cilia are quite short; but it may be seen that they elongate rapidly during the progress of cleavage so as to give the appearance of being pushed out slowly from within. The growth is sufficiently rapid almost to be seen at magnifications of 2,000 diameters—that is, one readily notes that they have increased in length in the course of a few seconds during which the attention has been fixed upon them. When mature, they are relatively long, but of unequal length. It is interesting to note that their combined length approximates twice the greater circumference of the spore, as is shown in Table I.

TABLE I.—*Relation of length of the cilia to the circumference of the spores (in microns)*

Length of shortest cilium.	Length of longest cilium.	Combined length of cilia.	Average length of cilia.	Greater circumference of spore.
19	31.5	50.5	25.25	24
16	34	50	25	24
22	27	49	24.5	25
16	33	49	24.5	26
21.5	27	48.5	24.25	24
21	25	46	23	27
14	29	43	21.5	24
15	28	43	21.5	21
10	31.5	41.5	20.75	24
17	24	41	20.5	22

In the absence of more definite information the evidence given in Table I might be used in support of the theory sometimes advanced that the cilia are of peripheral origin; but in the present instance at least it can be regarded only as an interesting correlation, since, as has already been pointed out, the cilia are put forth from the blepharoplast by a process of gradual elongation.

Each spore contains a single large central vacuole lying in contact with the nucleus and on the side toward the more pointed end of the spore (Pl. XLVI, fig. 4, 11).

The mitochondria are arranged in the zoospore at the periphery (Pl. XLVI, fig. 2). It is interesting to note that before cleavage furrows have pushed through the dividing protoplasm of the sporangium the mitochondria have already arranged themselves at what is to be the periphery of the future spore (Pl. XLV, fig. 3).

After coming to rest, the zoospore rounds up and undergoes certain changes preparatory to germination. Vacuoles develop (Pl. XLV, fig. 13), and karyokinetic nuclear division occurs (Pl. XLVI, fig. 15, 16, 17). One or usually two germ tubes develop (Pl. XLVI, fig. 3, 14, 17), forming a mycelium. The nuclei continue to divide within the spore and migrate into the mycelium, but divisions do not occur in the hyphæ until they have become mature (Pl. XLVI, fig. 3).

As the study of oogenesis is approached, it appears that both the antheridium and oogonium are multinucleate. The nuclei originate by karyokinesis in the parent hyphæ and migrate to the reproductive organs exactly as in the asexual stage. No divisions have been found to occur in either the antheridium or oogonium, although thousands have been sectioned and studied (Pl. XLV, fig. 8, and XLVIII, fig. 2, 3). After the requisite amount of material has been accumulated, the organs are cut off by cross walls. An eccentric cavity develops in the oogonium, so that the nuclei are arranged in a zone near the periphery (Pl. XLVIII, fig. 9). The vacuole then disappears; in its place there develops an area which takes the stain more densely than the surrounding cytoplasm, and a single nucleus comes to be within this area (Pl. XLVIII, fig. 8). In the meantime, the remaining egg nuclei undergo degeneration without division in the region where the wall of the oosphere is to appear (Pl. XLVII, fig. 2, 5, and XLVIII, fig. 5, 8). A receptive papilla forms on the antheridial side of the egg, and a passage way is opened from the antheridium through which a single nucleus and a considerable quantity of cytoplasm pass into the oosphere (Pl. XLVII, fig. 2, 5, 6, and XLVIII, fig. 8). The remaining antheridial nuclei degenerate (Pl. XLVII, fig. 2, 5, and XLVIII, fig. 8). The functional antheridial nucleus takes a position within the denser staining area at the center of the egg beside the egg nucleus and eventually fuses with it (Pl. XLVII, fig. 1, and XLVIII, fig. 5, 6, 7, 10).

There appears to be a certain amount of latitude as to the order in which some of the foregoing steps may occur. Sometimes the central

vacuole may persist till the degeneration of the supernumerary nuclei of the egg has reached an advanced stage (Pl. XLVII, fig. 2). Fertilization may be delayed till the degeneration of the nuclei is nearly complete (Pl. XLVII, fig. 5); or in exceptional cases it may occur even before the supernumerary nuclei have lost their nucleoli (Pl. XLVII, fig. 6). It is also apparent that the dense area at the center within which fusion occurs may arise before the degeneration of the nuclei (Pl. XLVII, fig. 6); or it may not appear till after fertilization has occurred (Pl. XLVII, fig. 2).

This body, which at once suggests a cœnocentrum, has been the subject of much speculation. Its presence in the species to be described is easily demonstrated. The functional nucleus of the egg takes a position within it where it is joined by the male nucleus, and the two fuse within it. Shortly after fusion it disappears, to be replaced by the lypoid material and stored food of the oospore. Critical study of the origin of the lypoid body, however, clearly demonstrates that the two are entirely distinct. The suggestion that the cœnocentrum-like body is the early stage in the formation of a food vacuole is not tenable in the case of this fungus, since that body is formed by the union of a large number of small lypoid granules which first develop outside the area in question, to coalesce later in the center of the spore (Pl. XLVIII, fig. 4, 6, 7, 10). On the other hand, it seems equally impossible to regard it as a true cœnocentrum originating from the centrosomes of the degenerating nuclei. The fact that these nuclei degenerate without previous division raises a certain, although not unsurmountable, element of doubt. The fact that it is not always present, even when degeneration has reached an advanced stage (Pl. XLVII, fig. 2), is significant, but the most conclusive proof comes from the fact that it may appear before degeneration has begun (Pl. XLVII, fig. 6). In Plate XLVII, figure 2, where the male nucleus has already entered the egg, the body in question is not present, and the egg nucleus remains at one side. It might be argued that the unusual position of the functional female nucleus at this stage is to be explained by the absence of the body and the consequent want of an attractive force to draw it to the center; in other words, that the mass under discussion is, in fact, a cœnocentrum. It seems more logical to the writer, however, to reason that the same force which causes the central vacuole to fill with denser protoplasm continues to act and that the accumulation of the denser material of the cœnocentrum-like body, the return of the female nucleus, and the approach of the male nucleus, as well as the subsequent accumulation of food material in the center of the spore, are but manifestations of its presence.

It has already been noted that in fertilization a considerable quantity of cytoplasm passes with the nucleus from the antheridium (Pl. XLVII, fig. 5). In preparations stained to show mitochondria it may be seen that this cytoplasm carries a mass of mitochondria which are more

closely clustered here than in the remaining contents of either the egg or the antheridium (Pl. XLVII, fig. 6). This dense clustering indicates that the presence of mitochondria in fertilization is not merely accidental and invites speculation as to their function. Their orientation at the periphery in the vegetative mycelium, and more especially in the zoospores, suggests that they may be responsive to light stimulus, and their accumulation in the presporangia and sex organs may indicate a nutritive function. The evident close philogenetic relationship of the fungus to algal forms perhaps supports the view that they are plastids, possibly degenerate chloroplasts. Their participation in fertilization raises a query as to whether they may not be charged with some part in the transmission of hereditary characters. A more novel and very interesting suggestion recently proposed to the author is that these bodies may be liquid crystals, and that, if this be true, a study of their origin and organization may lead to an understanding of the physical link which binds life to nonliving material; that in them we may discover, evolving from the inorganic and nonvital, that combination of physical and chemical properties with matter which characterizes life.

Following fertilization, food vacuoles appear in the cytoplasm (Pl. XLVIII, fig. 6, 7, 10) and then gather in a mass at the center (Pl. XLVII, fig. 3, and XLVIII, fig. 4). This crowds the fused nucleus from its position and leads to the development of a cytoplasmic zone about the central lypoid body (Pl. XLVI, fig. 12, and XLVII, fig. 3). In the meantime a thick spore wall has developed. There is at first a lighter zone between the cytoplasm and the wall (Pl. XLVI, fig. 19, and XLVII, fig. 3), but this gradually disappears, evidently by the shrinking and contraction of the wall (Pl. XLVI, fig. 12, 20).

The fused nucleus embedded in the cytoplasmic zone at first contains two nucleoli located at the poles (Pl. XLVII, fig. 3). These eventually disintegrate, but before doing so they migrate from the polar positions into the body of the nucleus (Pl. XLVI, fig. 5, *a*, *b*, *c*, 12, 20).

Another interesting phenomenon was observed in scores of cases—in the fused nucleus only and in material from but two lots, so that the work on this point can not be regarded as complete or as thoroughly established as is desirable. Owing to the pressure of other duties, however, the studies are not to be continued, and the observation is recorded here in the hope that it may be of value to other interested workers. During the progress of the changes of the nucleoli mentioned in the preceding paragraph, a single small body appears in contact with the nuclear membrane at one end (Pl. XLVI, fig. 5, *a*). Shortly following this, two bodies are present, one of which moves around to the opposite pole (Pl. XLVI, fig. 5, *b*, *c*, *d*, 19, 20). The position of the body suggests a centrosome.

Following the stages already discussed, there occurs a division of the nucleus (Pl. XLVII, fig. 7), giving rise to two nuclei which come to full

maturity (Pl. XLVI, fig. 18), promptly migrate to opposite sides of the cell, and undergo a second division, from which four nuclei arise (Pl. XLVII, fig. 4). Somewhat later a third division gives rise to eight nuclei. This is the greatest number observed in any of the material sectioned, but it is possible that additional divisions occur with the advancing maturity of the oospore.

The stage shown in Plate XLVII, figure 7, appears to be a reduction division, but it has not been possible to obtain the convincing evidence that would be afforded by chromosome counts. The fused nuclei are larger and differ in shape and in reaction to stain from the nuclei of other stages, while the two that result from the heterotypic division are similar in size and appearance to other nuclei. The interval between the first or heterotypic, and the second, or homotypic, division, while sufficiently long for the nuclei to become fully mature and to migrate to opposite sides of the cell, is relatively brief, so that the binucleate stage is seen much less frequently than the others.

It would be interesting to follow the history from the 8-nucleate stage of the oospore through germination, but the practical prosecution of such studies can be attempted wisely only after the conditions which induce germination are better worked out.

TAXONOMY OF THE FUNGUS

The characters of the fungus discussed in the preceding sections clearly place it in the Saprolegniaceae, but in none of the existing genera of that family. Among the systematic works dealing with the group that by Minden,[1] is the most recent. The system employed, which differs in some important respects from those of Schröter[2] and Fischer,[3] seems well conceived and logical and is so constructed as to provide readily a coordinate place for a new genus having the characters of the one to which the fungus under consideration is assigned. It will therefore be used in discussing the relationships of the organism.

Minden divides the Saprolegniaceae into two sections, according to the method in which the spores are liberated: Section A, in which all the zoospores of a sporangium escape through a common opening, and section B, in which they do not escape through a common opening. Section A, to which belongs the organism being treated, comprises subdivisions with diplanetic and monoplanetic spores. The diplanetic subdivision, with which we are especially concerned, consists of two groups. The first provides for Saprolegnia and Leptolegnia, where the zoospores are distributed for a swarm period immediately on liberation from the sporan-

[1] Minden, M. D. von. Pilze. *In* Kryptogamenflora der Mark Brandenburg, Bd. 5, Heft 3–4. 1911–12.
[2] Engler, Adolf, and Prantl, K. A. E. Die natürlichen Pflanzenfamilien . . . T. 1, Abt. 1, p. 96. Leipzig. 1897.
[3] Rabenhorst, Ludwig. Kryptogamen-Flora Deutschland, Oesterreich und der Schweiz. Aufl 2, Bd. 1, Abt. 4, p. 326. Leipzig. 1892.

gium. They then come to rest, undergo metamorphosis, and emerge as more or less bean-shaped, biciliated zoospores for a second and more prolonged period of motility. This group presents a pronounced condition of diplanetism.

The second group, on the other hand, comprises Achlya and Aphanomyces, forms that present a condition of what may be called "reduced diplanetism." Here the first swarm period is reduced to a simple migration from the sporangium. It occurs after the spores are cleaved and is followed by metamorphosis from which the zoospores emerge, as in the first group, for a prolonged period of motility.

The fungus under consideration seems to present a third and hitherto undescribed type of diplanetism, in which the first motile period consists in the migration of the entire uncleaved sporangium and its contents from the presporangium. This type of egress is new. In all related forms previously described the spores are differentiated before migration. The distinction seems sufficiently important to justify its recognition as of generic rank. The uncleaved protoplasm rather than the differentiated spore migrates. The process of metamorphosis is eliminated and the spores that arise from cleavage have the form characteristic of the second motile period in the other genera mentioned.

The tendency in the series outlined has been toward monoplanetism, but it hardly seems probable that such a condition has arisen in this manner, since Pythiopsis, the monoplanetic genus of the subdivision, has eliminated metamorphosis and the second motile period rather than the first, giving the entire interval of locomotion to its more or less egg-shaped spores, the form type characteristic of the first motile period of genera like Saprolegnia.

It will be noted that for taxonomic consideration the body previously termed a "presporangium" has been regarded as closely analogous to a sporangium. Some may prefer to discard the prefix and apply the term "sporangium" to the portion of the hypha cut off for purposes of spore production, giving another name to the thin-walled organ in which the spores arise. It has seemed to the author, however, that the name "sporangium" should be applied to the organ in which the spores are differentiated, and that the term "presporangium," while distinctive, is at the same time clear and accurate, conveying a true idea of the function of the body to which it has been applied.

In selecting a name for the fungus an effort has been made to choose one which is descriptive of some distinctive character. From the fact that the sporangium flows out from the presporangium and that its wall is so delicate as to be almost invisible, the name "*Rheosporangium aphanidermatus*" has been chosen. Its technical description is as follows:

RHEOSPORANGIUM, new genus.

Mycelium aerial or aquatic, well-developed, nonseptate, branched. Reproduction by zoospores under aquatic conditions and by oospores. Terminal, enlarged, more or less distorted mycelial-like prezoosporangia cut off from the ends of hyphæ. Sporangia thin-walled, normally escaping from the presporangium through a terminal rupture, cleaving into zoospores.

Rheosporangium aphanidermatus, n. sp.

Vegetative mycelium white, in water hyaline, nonseptate except in fructification, branched, finely granular, frequently exhibiting pronounced protoplasmic streaming. Young hyphæ varying in width from 2.8 to 7.3μ, averaging from 4 to 6μ, but frequently becoming wider as fructification approaches. Presporangia developing by the enlargement of terminal portions of hyphæ, unbranched or irregularly clavately to normally branched, length varying from less than 50 to more than 1,000μ, width from 4 to 20μ, distal portion usually unbranched and tapering to a rounded end. Sporangia with nearly invisible, flexible, membranous walls escaping from the distal end of the presporangia or, rarely, from that of one of the branches, becoming spherical on release, varying in diameter according to the presporangia, at once cleaving into zoospores. Zoospores escaping by the rupture of the sporangia, plano-convex, with a single central vacuole, and on the flattened side a sinus, from the bottom of which the two cilia of unequal length arise. Average size, 12 by 7.5μ. Oogonia terminal, spherical, 22 to 27μ in diameter. Antheridia terminal or intercalary, suborbicular, becoming cylindric or broadly clavate, average dimensions, 9 to 11 by 10 to 14μ. Oospores single, smooth or contoured, average diameter, 17 to 19μ.

The following illustrations are reproduced from camera-lucida drawings of fixed and stained material, except when otherwise stated.

PLATE XLIV

Rheosporangium aphanidermatus:

Fig. 1.—Cleavage of the sporangium into zoospores within the wall of the presporangium. ×2,000.

Figs. 2, 3, 4, 5, 8, 9, 11.—Nuclear divisions in the mycelium. Fig. 9, ×2,000, others ×3,000.

Fig. 6.—Portion of presporangium showing rupture at tip and the initial stage of sporangium egress. ×2,000. See also figure 10.

Fig. 7.—Maturing presporangium. ×250.

Fig. 10.—Later stage of sporangium egress from a branch of the presporangium. ×2,000. See also figure 6.

Fig. 12.—Vegetative mycelium; living. ×500.

Fig. 13.—Section of nearly mature presporangium, showing development of large vacuoles. ×2,000. See also Plate XLVIII, figure 1.


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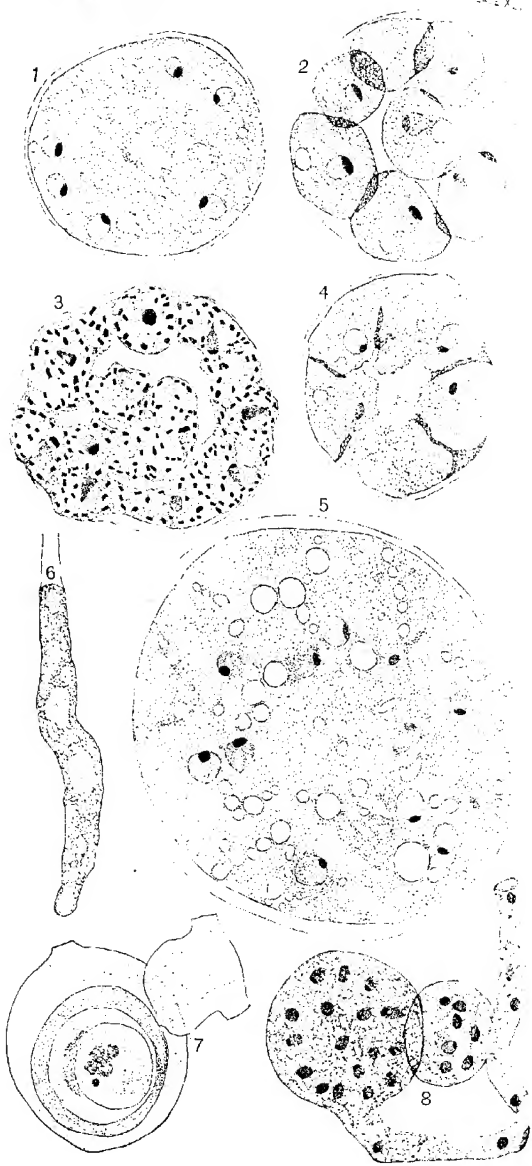
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PLATE XLV

Rheosporangium aphanidermatus:

Figs. 1, 2, 3, 4, 5.—Sections of sporangia showing various stages of cleavage into zoospores. Figure 3 shows mitochondria and, at the periphery, fragments of cilia. ×2,000.

Fig. 6.—Segment of mycelium showing accumulation of cytoplasm characteristic of old aquatic cultures; living material. ×750.

Fig. 7.—Oospore within the old oogonial wall, antheridial wall attached. From typical unstained, living material produced in aquatic culture. ×1,500.

Fig. 8.—Young oogonium and antheridium not yet cut off from the parent hyphæ. Fixed and stained, but unsectioned. ×2,000. See also Plate XLVIII, figures 2 and 3.

PLATE XLVI

Rheosporangium aphanidermatus:

Fig. 1.—Section of presporangium showing nuclei and mitochondria. ×2,000.

Fig. 2.—Section of zoospore showing mitochondria. ×2,000.

Fig. 3.—Advanced stage of zoospore germination. ×2,000.

Fig. 4.—Section of zoospore showing position of central vacuole and nucleus. ×2,000. See also figure 11.

Fig. 5.—Stages in the preparation of the fused nucleus of the oospore for division. ×2,000.

Fig. 6.—Zoospore showing position of sinus in side. ×2,000.

Fig. 7.—Section through zoospore showing blepharoplast and sinus. ×2,000.

Fig. 8.—Mycelium showing nucleus and mitochondria. ×2,000.

Figs. 9, 10.—Sections of zoospores showing blepharoplast and attachment of cilia ×2,000.

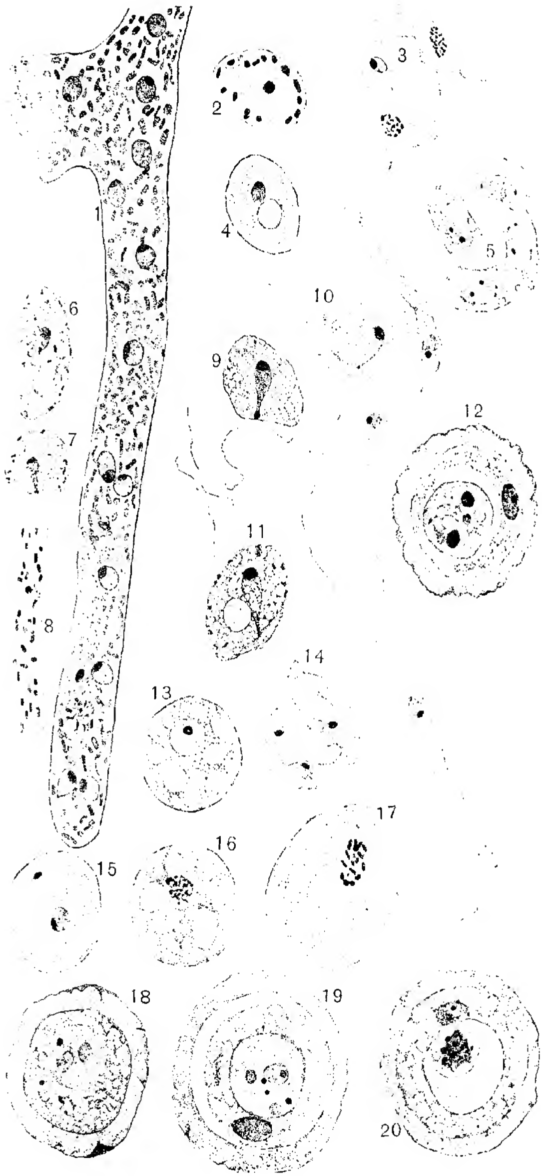
Fig. 11.—Zoospore showing orientation of the various structures. ×2,000.

Fig. 12.—Maturing oospore showing fused nucleus, large central food body, and the wall, which is unusually deeply contoured. ×2,000.

Figs. 13, 14, 15, 16, 17.—Stages in zoospore germination. ×2,000. See also figure 3.

Fig. 18.—Section of maturing oospore. First division of fused nucleus completed. ×2,000.

Figs. 19, 20.—Maturing oospores showing usual type of wall and the fused nucleus in preparation for division. ×2,000. See also figures 5 and 12.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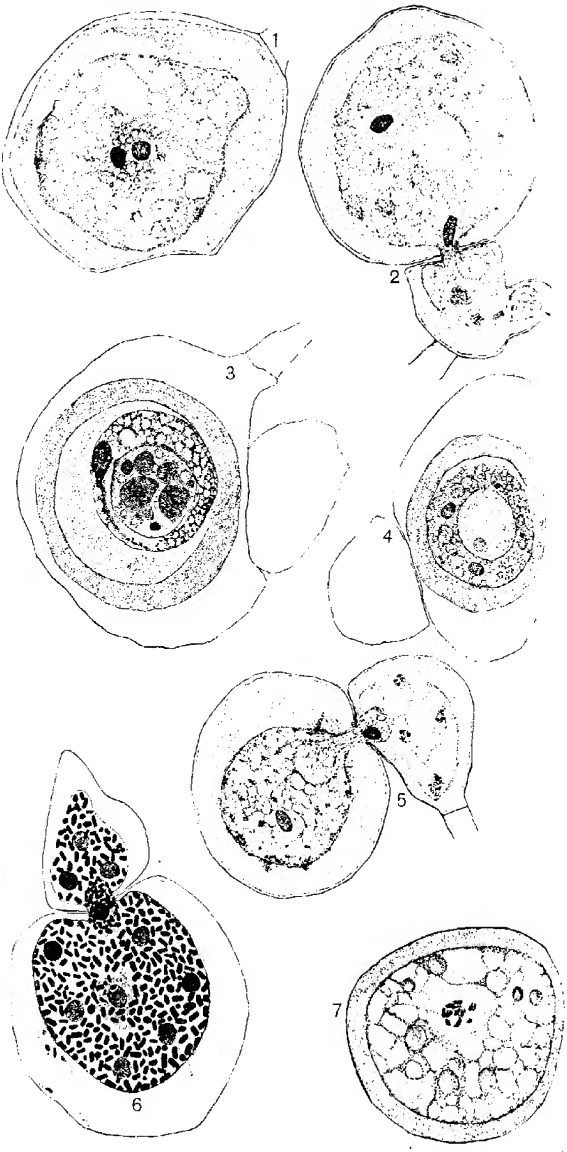
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PLATE XLVII

Rheosporangium aphanidermatus:

Fig. 1.—Fertilized egg showing the two functional nuclei nearing juxtaposition. ×2,000.

Fig. 2.—Fertilization taking place before the central vacuole of the egg has entirely disappeared. Disintegration of supernumerary nuclei well advanced. ×2,000.

Fig. 3.—Maturing oospore showing the large central food body and the fused nucleus with two polar nucleoli. ×2,000.

Fig. 4.—Oospore after the second nuclear division. ×2,000.

Fig. 5.—Fertilization: Cytoplasm and functional nucleus passing from antheridium to oosphere. ×2,000.

Fig. 6.—Fertilization occurring before degeneration of the supernumerary nuclei. Mitochondria shown passing into the oosphere with the nucleus. ×2,000.

Fig. 7.—First division of the fused nucleus in the oospore. The central food body, which lies immediately beneath the nucleus, is not shown. ×2,000.

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PLATE XLVIII

Rheosporangium aphanidermatus:

Fig. 1.—Section of presporangium showing intermediate stage of vacuolization. ×2,000. See also Plate XLIV, figure 13.

Fig. 2, 3.—Developing oogonia and antheridia. ×2,000. See also Plate XLV, figure 8.

Fig. 4.—Oospore after nuclear fusion showing accumulation of food material at the center. ×2,000. See figures 6, 7, and 10 for earlier stages of food body.

Fig. 5.—Fertilized egg showing functional nuclei within the deeper stained central portion. ×2,000.

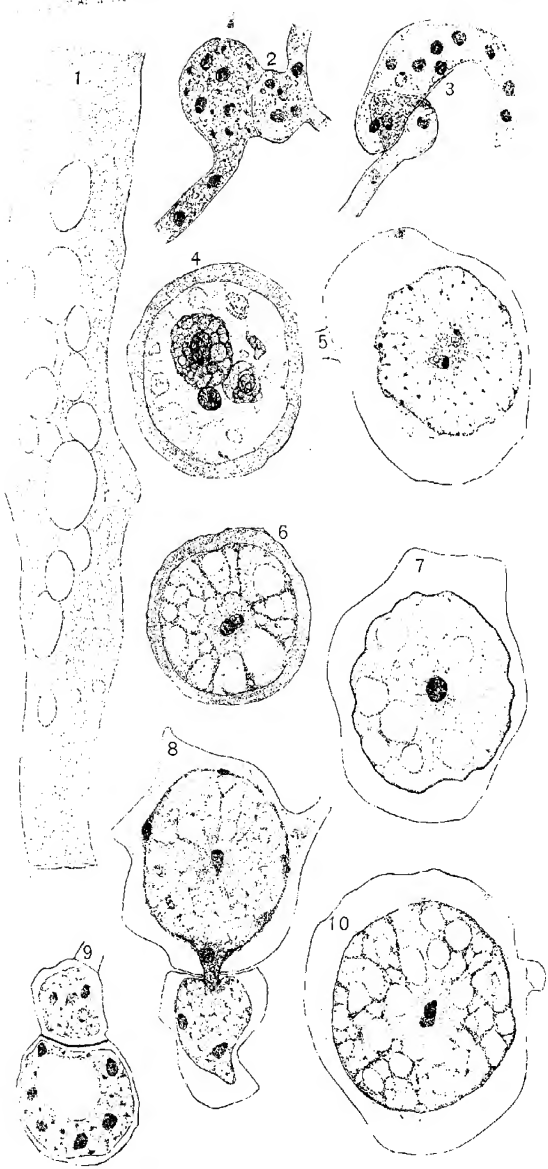
Fig. 6.—Fertilized egg showing functional nuclei in juxtaposition within central mass. Food bodies (decolorized) appear as vacuoles in the cytoplasm. Note the thickness of the wall, unusual at this stage. ×2,000. See also figure 10.

Fig. 7.—Fertilized egg showing nucleus after fusion within the deeper stained central portion and position of decolorized food bodies. ×2,000.

Fig. 8.—Typical fertilization: Functional egg nucleus in deeply staining center of oosphere; others degenerating at the periphery. Functional antheridial nucleus entering the oosphere; others degenerating in the antheridium. ×2,000.

Fig. 9.—Young oogonium and antheridium showing the central vacuole and the peripheral arrangement of the nuclei. ×2,000.

Fig. 10.—Fertilized egg: Oospore wall formed but not thickened, functional nuclei in juxtaposition within central mass, and food bodies in surrounding cytoplasm. ×2,000. See also figure 6.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HEREDITY OF COLOR IN PHLOX DRUMMONDII[1]

By ARTHUR W. GILBERT,

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INTRODUCTION

Workers in the field of heredity, stimulated by Gregor Mendel's classical experiments, are attempting to prove or disprove the hypothesis, which is now quite generally accepted, that plants and animals are composed of distinctly heritable units, now called "unit characters." After the presence of these units has been demonstrated, the next problem is to determine what these units are in different plants and animals, and their exact mode of inheritance.

These experiments were planned to solve these problems with *Phlox drummondii*.

METHODS OF PROCEDURE

This plant was chosen because its flowers have a wide range of colors, it is easy to grow both in the greenhouse and out of doors, and crossing is not difficult.

Commercial seed was purchased and the different varieties grown and self-fertilized for three years, so as to be sure of pure types. The varieties used in these experiments were found to breed true for three years and are assumed to be pure.

The crossing was done in the ordinary way, great care being exercised at all times to prevent the admission of foreign pollen. All flowers were carefully bagged with small oiled bags, which were tied as tightly around the stem as the growth of the plant would permit. The parents were self-fertilized each year and grown alongside of the F_1 and F_2 hybrids.

Notes were carefully made of the color of the flowers, according to the nomenclature in Répertoire de Couleurs.[2] Inasmuch as the color fades rapidly in intense sunlight, the descriptions were made soon after the flower had first opened. These colors are described in the tables by naming the number of the page and the shade which corresponds nearest to it.

[1] Paper No. 55, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

[2] The following color book was used as a standard basis of comparison of the colors: Société Française des Chrysanthémistes. Répertoire de Couleurs. 82 p., illus., 3 pl. (2 col.), and 365 col. pl. in 2 portfolios. Paris, 1905.

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Four varieties of phlox are employed in these experiments: Eclipse, Large Yellow, Coccinea, and Carnea. The Carnea variety was used as the male parent in all of the crosses.

Flowers that were practically colorless were recorded as "white" in the tables, but, as the color plates show, a very slight amount of color was present in many of them. They are probably not pure albinos.

THE NATURE OF COLOR

The colors of plants are due to constituents which are either colored themselves or act upon other substances to produce color in them. All of the cells of the plant contain these substances, with the possible exception of meristematic or rapidly growing tissue. Plant pigments may be divided into two classes with reference to their location in the cell: Chromoplast colors and cell-sap colors. The first class includes green, usually yellow and orange, and occasionally red; the second class, mostly red, blue, and violet.

Buscalioni and Traverso distinguish the following classes:

1. Green (chloroplasts).
2. Yellow and orange (chromoplasts).
3. White (colorless, made white by air in intercellular spaces).
4. Red.
5. Violet and lilac.
6. Blue (4 to 6 anthocyan pigments in solution).
7. Brown (tannin probably concerned).

Various other colors are supposed to be due to the mixing or modification of the pigments referred to. The black spots and stripes on the flowers of the broad bean, for instance, are evidently due to violet pigment, since the stripes at first are violet.

The yellow color of flowers is due in most cases to chromoplasts containing yellow anthoxanthin; but, rarely, yellow is a cell-sap color, as, for example, in *Mirabilis longiflora* and the yellow parts of a white dahlia. In the latter case there is a transition to red cell sap that establishes the close relationship of these two colors.

In yellow beets, also, there is yellow cell sap, probably closely related to the red sap color of the beet.

The yellow-brown colors found in seeds and fruits especially are considered to be largely due to tannin, which is itself colorless but readily produces color through the action of carbon dioxid.

Reds are usually cell-sap colors. Chrome reds and brick reds are exceptions. The tomato and the carrot, for instance, have red chromoplasts.

The blue and the purple color substances in flowers are dissolved in the cell sap and are distinguished for the most part from the plastid colors by being insoluble in ether, xylol, benzol, chloroform, carbon disulphid, and similar solvents, but are soluble in water or alcohol.

PLASTID COLOR SUBSTANCES

There are found in most plant cells lying in the cytoplasm outside of the nucleus small bodies called "chromatophores." In embryonic cells and growing points these chromatophores are colorless and highly refractive, and this condition may be retained until the cells reach maturity. Ordinarily, however, these colorless chromatophores attain a further development as chloroplasts, leucoplasts, or chromoplasts.

CHLOROPLASTS

In parts of the plant which are exposed to the light, the chromatophores usually develop into chlorophyll bodies of flattened ellipsoidal shape and are scattered in numbers in the parietal cytoplasm of the cells. These granules contain two pigments, green and yellow, the former predominating often to the complete exclusion of the latter. The yellow pigments of the chloroplasts are collectively termed "xanthophyll." The green and yellow pigments may be separated from each other, and each can be readily seen by placing green leaves, which have been previously boiled in water, in alcohol and adding benzol. When this solution is shaken and then allowed to stand, the benzol will rise to the surface as a green solution, leaving the alcohol yellow.

CHROMOPLASTS

The chromoplasts of most flowers and fruits arise either directly from the rudiments of colorless chromatophores or from previously formed chloroplasts. The color of the chromoplasts varies from yellow to red, according to the predominance of yellow xanthophyll or orange-red carotin. The name "carotin" has been derived from the carrot (*Daucus carota*), in the roots of which it is particularly abundant. Carotin is practically identical with the so-called "chrysophyll" found in the chloroplasts.

It will be seen that there is a very close relationship between the chloroplasts and chromoplasts and the green and yellow colors found in them. In general, however, it may be said that the yellow color in certain roots, flowers, and fruits is due to the yellow pigments of the chromatophores.

CELL-SAP COLOR SUBSTANCES

During the process of metabolism the plant cell manufactures other color substances which are not combined with the protoplasm, but which are contained in the cell sap, or liquid of the cell. These substances, unlike other plastid colors, are insoluble in xylol, ether, or similar solvents, but are soluble in water and alcohol, which afford a means of separating them from the plastid colors. These cell-sap pigments may

occur in cells free from plastids or in the vacuoles of cells containing plastids, but not associated with them as a part of the organized body or plastid. These pigments have one property in common with the chromophyll substances—i. e., with alkalies, potassium cyanid, and sodium phosphate they assume some shade of green. They are distinguished, however, by the fact that the colors are markedly affected by acids and alkalies and by iron salts. The fact that these substances are so sensitive to reagents probably accounts for the various shades and tints characteristic not only of flowers but of leaves as well. Kraemer has observed in the germinating kernels of black Mexican sweet corn that even in contiguous cells the constituents associated with the dye vary to such an extent that the pigment in one cell is colored reddish, in another bluish green, and in another purplish.

COLOR HEREDITY

It will be seen that the nature of color is very complex, and consequently its heredity is equally so. Mendel's studies of peas and many other similar plants seem now comparatively simple, because they deal with characters which are easily distinguishable with little reaction of one upon the other. Each unit was found to be separately heritable and could quite easily be traced from generation to generation. Not so with colors, the units of which are obscure; being chemical in nature, reactions of various sorts occur, making experimentation difficult.

RESULTS OF CROSSING

SERIES I

POLLEN PARENT.—A pale rosy-pink variety known commercially as Carnea. The color corresponds to shade 1 on page 129 of the Repertoire de Couleurs, designated hereafter as "shade 129-1." This variety has a white eye—that is, the center of the flower is white and arranged as a distinct pattern (Pl. C).

SEED PARENT.—A violet-purple variety (192-3) known in commerce as Eclipse. This variety had a dark eye—that is, the center of the flower had a denser, deeper color than the remainder of the flower, but without any particular pattern.

FIRST AND SECOND GENERATION HYBRIDS.—The F_1 hybrids were unlike either parent. They were Tyrian rose (155-4), with a dark eye. The color of the F_1 hybrids immediately suggested the presence in the parents of complementary color factors, which were united to produce something different from either parent.

All plants having similar colors were placed together in groups and the numbers recorded. Table I gives the results of the F_2 hybrids grouped as accurately as possible in this way.

TABLE I.—*Color of the progeny of two varieties of Phlox drummondii, the Eclipse and the Carnea*

Parent or progeny.	Phenotypic formula.	Phenotype.	Field count.	Calculated.	Ratio.
Seed parent: *Phlox drummondii*, var. Eclipse.	EBrI	Violet-purple (192–3), dark eye.			
Pollen parent: *Phlox drummondii*, var. Carnea.	ebRi	Pale rosy pink (129–1), white eye.			
F_1 generation	EeBbRrIi	Tyrian rose (155–4), dark eye.			
F_2 generation:					
Alternative factors—					
E B R I	EEBBRRII	Reddish violet (180–4), dark eye (Pl. C, fig. *A*).	204	171. 81	81
E B R i	EEBBRRii	Bright violet (198–4), dark eye (Pl. C, fig. *B*).	16	57. 23	27
E B r I	EEBBrrII	Bright violet-purple (190–3), dark eye (Pl. C, fig. *C*).	41	57. 23	27
E B r i	EEBBrrii	Dauphin's blue (203–2), dark eye (Pl. C, fig. *D*).	39	19. 09	9
E b R I	EEbbRRII	Crimson carmine (189–3), dark eye (Pl. C, fig. *E*).	58	57. 23	27
E b R i	EEbbRRii	Pale lilac-rose (130–1), dark eye (Pl. C, fig. *F*).	12	19. 09	9
E b r I	EEbbrrII	White, pigmented (pink) eye (Pl. C, fig. *G*).	1	19. 09	9
E b r i	EEbbrrii	White (Pl. C, fig. *H*)	(a)	(a)	(a)
e B R I	eeBBRRII	Magenta (183–3), white eye (Pl. C, fig. *I*).	71	57. 23	27
e B R i	eeBBRRii	Pale light lilac (187–1), white eye (Pl. C, fig. *J*).	6	19. 09	9
e B r I	eeBBrrII	Pale purple, white eye (Pl. C, fig. *K*).	17	24. 45	12
e B r i	eeBBrrii				
e b R I	eebbRRII	Tyrian rose (155–3), white eye (Pl. C, fig. *L*).	10	19. 09	9
e b R i	eebbRRii	Pale lilac-rose (130–1), white eye (Pl. C, fig. *M*).	65	6. 36	3
e b r I	eebbrrII	White (Pl. C, fig. *H*)	3	14. 84	7
e b r i	eebbrrii				
Total			543		256

a See explanation of white below.

EXPLANATION OF SYMBOLS AND FACTORS IN TABLE I

The results from the F_2 hybrids suggest that the following factors are present in the parents. These factors have been represented by symbols as indicated below.

"E." A factor for dark eye color producing a denser coloration at the center of the flower, or eye. E does not act in the absence of the color factors B, R, or I (Pl. C, fig. *G*), but is effective if any one of them is present.

"e." The absence of an eye factor produces a white eye. The latter, unlike the dark eye, seems to have a distinct pattern (Pl. C, D, E).

"B." Presence of blue pigment. This is not a pure blue, but contains red, producing purple. The blue and red do not become dissociated, but are inherited together throughout the series. This red seems to bear no relationship to the red (R) brought in by the pollen parent, Carnea.

"b." Absence of blue.

"R." Presence of red. This is a distinct red factor which is inherited separately from all others. The absence of the factors for color, B and R, produces white.

"r." Absence of the red brought in by the pollen parent.

"I." An intensifying factor which determines the degree of pigmentation. This seems to affect the red only (Pl. C, fig. *A*, *B*). This factor evidently carries with it a considerable amount of red. Possibly the apparent intensification of the reds is nothing more than the addition of more red—that is, R and I may each represent distinct red factors. Plate C, figure *B*, shows a bright violet of phenotypic formula EEBBRRii, in which the intensifying factor I is absent. But in a similar series (EEBBRRII), Plate C, figure *A*, where it is present, much additional red is devel-

oped. The same is true for all other series containing I or i. Futhermore, when neither B nor R is present, as in Plate C, figure *G*, red pigment develops in the eye, probably owing to the factor I, bringing in the red, and the factor causing a dark eye. E acts upon I to produce the pink (red) eye.

I seems to have affected the reds (R), but not the blues nor the red associated with the blue making purple, represented by factor B. For example, the plants with the phenotypic formula eeBBrrii (Pl. C, fig. *K*) seem to be the same, the I having nothing to act upon, E or R, to cause more red to appear. The same is true of EEbbrrIi and eebbrrii (Pl. C, fig. *H*); I has no effect, and both appear as white.

The Observed and Calculated Ratios

The 543 plants that comprised the F_2 generation were divided as accurately as possible into groups, each group containing plants which were similar in appearance. This was done long before any explanation was found to account for their inheritance. Therefore, the writer was not prejudiced in the slightest degree in making his selections. This division of this population into groups was exceedingly difficult. The boundaries of the groups were not clear-cut, and very many border plants were found which were not easy to classify. This difficulty of classification may account for some of the differences that are seen between certain observed and calculated ratios. These are not so serious, however, to the man who has worked with the plants and tried to classify them. He is surprised not so much at the differences but at the nearness to theoretical ratios in most cases. For example, the writer found it very difficult to divide a certain number of bluish or purplish plants into their proper groups. The extremes of these groups could be readily recognized, but there were very many plants which might, so far as observation was concerned, go as well into one group as another. But the writer made the grouping as correctly as possible, not knowing what the interpretation would be. This accounts for the small number of plants in type B (Pl. C) and the large number in type A (Pl. C). There were many plants which might be equally well classed in either group. Other differences may be accounted for in this way.

The writer is by no means certain that all of the differences between observed and calculated ratios can be thus accounted for. There may be present linkage or repulsion, but neither has been detected as yet. The interpretation of the observations is considered merely tentative. Future crossings may modify it in many ways.

The dearth of whites may perhaps be accounted for. The plants with white flowers in this series were noticeably the weakest, and inasmuch as many plants died during the prosecution of the experiment, it is likely that a considerable proportion of these were whites.

The weakness of white-flowered plants is a common observation among gardeners. Hottes, for example, says that white varieties of the gladiolus are so weak that it is almost impossible to propagate them.

If the classes are grouped together, the individual differences tend to disappear. For instance, if all of the plants with dark-eyed flowers (E)

are put in one class and the white-eyed ones (e) in another class, the results will be as follows:

	E	e
Observed	373	170
Calculated	407.2	135.8

A slight discrepancy occurs because the whites fall into two classes, some supposed to have E and others e. The total number of whites are split up into classes arbitrarily for the calculation. The same was done in a few other cases.

Similarly, if the other dominants and recessives are brought together, the results are the following:

	E	e	B	b	R	r	I
Observed	373	170	404	139	442	101	399
Calculated	407.2	135.8	407.2	135.8	407.2	135.8	407.2

	i	Dominants.	Recessives.
Average of all	144	404.5	138.5
	135.8	407.2	135.8

SERIES II

POLLEN PARENT.—The same variety, Carnea, which was used as pollen parent in Series I and III.

SEED PARENT.—A bluish lilac variety known in commerce as Coccinea. This is a dark-eyed variety, the center of the flower being more dense than the remainder of the flower, but without any color pattern (Pl. D).

TABLE II.—*Color of the progeny of two varieties of Phlox drummondii, the Coccinea and the Carnea*

Parent or progeny.	Phenotypic formula.	Phenotype.	Field count.	Calculated.	Ratio.
Seed parent: *Phlox drummondii*, var. Coccinea.	EBr	Bluish lilac (138–1), dark eye.			
Pollen parent: *Phlox drummondii*, var. Carnea.	eBr	Pale rosy pink (129–1), white eye.			
F_1 generation	EeBbRr	Carmine purple (136–2), dark eye.			
F_2 generation: Alternative factors— E B R	EEBBRR	Crimson carmine (119–3), dark eye (Pl. D, fig. *A*).	58	48.52	27
E B r	EEBBrr	Bluish lilac (138–1), dark eye (Pl. D, *B*).	18	16.17	9
E b R	EEbbRR	Rosy white (8–1), dark eye (Pl. D, fig. *C*).	5	16.17	9
E b r	EEbbrr	White, dark (pink) eye (Pl. D, fig. *D*).	1	5.39	3
e B R	eeBBRR	Rosy magenta (119–1), white eye (Pl. D, fig. *E*).	19	16.17	9
e B r	eeBBrr	(Perhaps some of the whites come in this class).	(a)	(a)	(a)
e b R	eebbRR	Bright rose (128–3), white eye (Pl. D, fig. *F*).	7	5.39	3
e b r	eebbrr	White {eeBBrr / eebbrr} (Pl. D, fig. *G*).	7	5.39	4
Total			115		(4

a See below.

EXPLANATION OF SYMBOLS AND FACTORS IN TABLE II

All of the second-generation hybrids of this series contain more red than the corresponding hybrids of the former series. This additional red unquestionably comes from the Coccinea parent. There are not sufficient data to determine what this additional red is and its entire behavior in heredity.

The factors E, e, B, b, R, and r have a similar significance to that in Series I, although they are evidently not exactly the same. Plants in Series I and II having the same phenotypic formula are not identical, as may be seen from the illustrations. This indicates that the factors of the two series are not identical.

OBSERVED AND CALCULATED RATIOS

The calculated and observed ratios correspond very closely in this series. This is the more evident when the relatively large number of classes and the small number of individuals are taken into account. Grouping the data as before gives the following results:

	E	e	R	r	Pigmented	White
Observed	82	33	89	26	108	7
Calculated	86.3	28.7	86.3	28.7	107.8	7.2

SERIES III

POLLEN PARENT.—The same variety, Carnea, as used in Series I and II.

SEED PARENT.—A yellow variety known commercially as Large Yellow. The pigmentation is more dense at the center eye than at other parts of the flower (Pl. E).

TABLE III.—*Color of the progeny of two varieties of Phlox drummondii, the Large Yellow and the Carnea*

Parent or progeny.	Phenotypic formula.	Phenotype.	Field count.	Calculated.	Ratio.
Seed parent: *Phlox drummondii*, var. Large Yellow.	ErY	Cream yellow (30–3), dark eye.			
Pollen parent: *Phlox drummondii*, var. Carnea.	eRy	Pale rosy-pink (129–1), white eye.			
F_1 generation	EeRrYy	Rose Neyron red (119–2), dark eye.			
F_2 generation: Alternative factors—					
E, R, Y	EERRYY	Deep lilac rose (153–2), yellow (dark) eye (Pl. E, fig. *A*).	26	30.69	27
E, R, y	EERRyy	Pale pink (135–1), dark eye (Pl. E, fig. *B*).	5	13.23	9
E, r, Y	EErrYY	Cream yellow (30–4), dark eye (Pl. E, fig. *C*).	9	13.23	9
E, r, y	EErryy	White, dark eye (Pl. E, figs. *D* and *E*).	10	4.41	3
e, R, Y	eeRRYY	Lilac purple (160–2), white eye (Pl. E, fig. *F*).	15	13.23	9
e, R, y	eeRRyy	Pale reddish lilac (131–1), white eye (Pl. E, fig. *G*).	6	4.41	3
e, r, Y	eerrYY	White (Pl. E, fig. *H*)	23	5.88	4
e, r, y	eerryy	White (Pl. E, fig. *H*)			
Total			94		64

EXPLANATION OF SYMBOLS AND FACTORS IN TABLE III

In addition to the factors already mentioned, we have a factor for yellow (Y), which acts only in the presence of the eye factor (E). Wherever Y is present and E is absent, only white occurs.

OBSERVED AND CALCULATED RATIOS

The observed and calculated ratios are again equal, with the exception of the preponderance of white, which is unaccounted for.

SUMMARY

PLANT COLORS IN PHLOX DRUMMONDII.—(1) White is due to the absence of pigment and to the reflection of light from the cells. (2) Green color is caused by the presence of a green pigment in the chlorophyll. (3) Yellow, cream, and related colors are due to a yellow pigment either associated with green in the chloroplasts or found alone in the chromoplasts; generally the latter. Yellow may sometimes come from the cell sap. (4) Red color may under certain circumstances be due to the presence of that pigment in the chromoplasts, but is ordinarily a cell-sap color. (5) Most of the remaining colors, purple, blue, generally red, pink, etc., are due to pigments in the cell sap. (6) Many of the colors and shades found in flowers are the result of both plastid colors and cell-sap colors acting together in various proportions. (7) Certain of the denser plastids or cell-sap colors may cover up the more delicate colors so that they can not be seen. (8) Finally, the color in the cell sap may be due to the relative presence of a non-nitrogenous chemical substance, anthocyanin. This is blue in alkaline and red in an acid-reacting cell sap and, under certain conditions, also dark red, violet, dark blue, and even blackish blue. Anthocyanin can be obtained from the supersaturated cell sap of a number of deeply colored parts of plants in a crystalline or amorphous form. Blood-colored leaves, such as those of the copper beech, owe their characteristic appearance to the united presence of green chlorophyll and anthocyanin. The different colors of flowers are due to the varying color of the cell sap, to the different distribution of the cells containing the colored cell sap, and also to the combinations of dissolved coloring matter with the yellow, orange, and red chromoplasts and the green chloroplasts. There is occasionally found in the cell sap a yellow coloring matter known as "xanthein," nearly related to xanthophyll, but soluble in water.

COLOR INHERITANCE IN PHLOX DRUMMONDII.—The following unit characters were found in the four varieties of *Phlox drummondii* that were used in these experiments: (1) A dark eye factor producing a dense coloration at the center of the flower. This was dominant over its absence, the white eye, which was exhibited in more or less of a definite pattern. (2) A blue factor. (3) A red factor. (4) An intensifying factor which determines the degree of pigmentation of the reds. (5) A yellow factor which acts only in the presence of the eye factor.

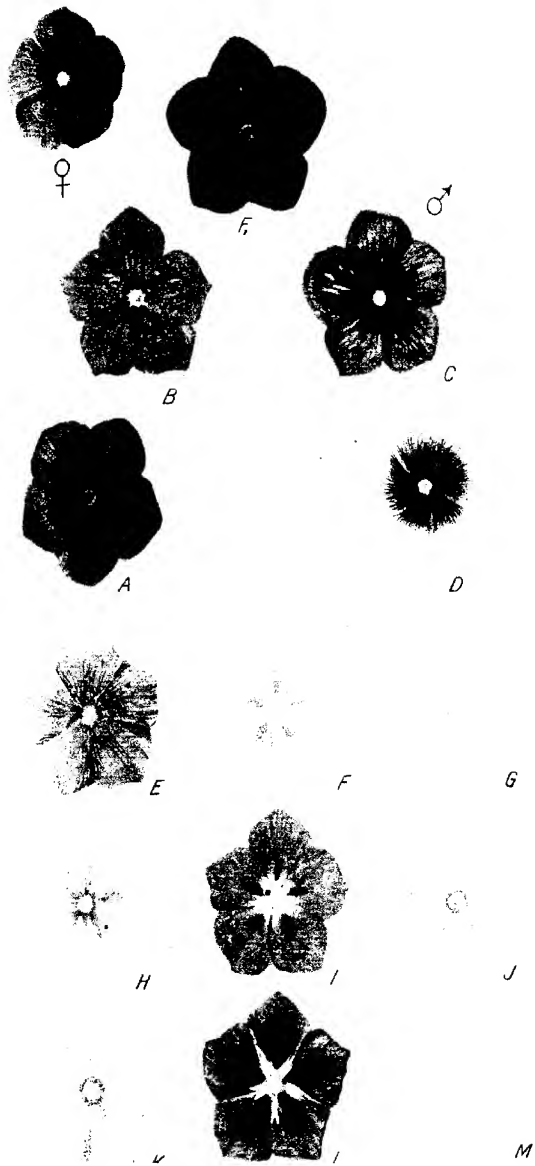
The reds and blues are cell-sap colors, and the yellow is due to the presence of yellow chromoplasts.

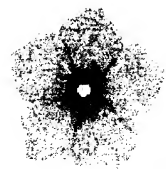
Studies of this nature will eventually lead to a time when color and color inheritance are sufficiently understood and controlled to be of great commercial value to the florist or grower of ornamental plants.

PLATE C

Phlox drummondii:

Fig. 1 ♀—Seed parent, Eclipse variety.
Fig. 1 ♂—Pollen p—arent, Carnea variety.
Fig. 1 F_1—First-generation hybrid between Eclipse and Carnea.
Fig. 1 A to M—Second-generation hybrids between Eclipse and Carnea.


♀
F1
♂
B
C
A
D
E
F
G
H
I
J
K
I
M

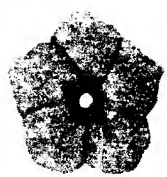


♀

♂

F,

A



B

C

D

E

F

G

PLATE *D*

Phlox drummondii:

FIG. ♀—Seed parent, Coccinea variety.
FIG. ♂—Pollen parent, Carnea variety.
FIG. F_1—First-generation hybrid between Coccinea and Carnea.
FIG. A to G—Second-generation hybrids between Coccinea and Carnea.

PLATE E

Phlox drummondii:

FIG. ♀—Seed parent, Large Yellow variety.
FIG. ♂—Pollen parent, Carnea variety.
FIG. F_1—First-generation hybrid between Large Yellow and Carnea.
FIG. A to H—Second-generation hybrids between Large Yellow and Carnea.

♀

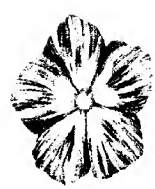
♂

F,

C

A

B



D

F

E

G

H

ASPARAGUS-BEETLE EGG PARASITE

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INTRODUCTION

On May 23, 1909, a minute chalcidid parasite was reported from Concord, Mass., by Messrs. C. W. Prescott and J. B. Norton, of the Bureau of Plant Industry, where, according to information received from Mr. Prescott, the insect was observed devouring the contents of the eggs of the asparagus beetle (*Crioceris asparagi* L.). Later, on June 2, Dr. H. T. Fernald found the same parasite at Amherst, Mass. The species was referred to the Bureau of Entomology and was determined by Mr. J. C. Crawford, of the United States National Museum, as being new to science, and was accordingly described as *Tetrastichus asparagi* Cwfd. (1).[1] In July of that year and later, in August, Dr. Fernald published short articles on this species. Since the asparagus beetles have never been carefully studied throughout their life history, the fact that the parasite had been recorded was overlooked. In an earlier article, however, published in 1869, Riley and Walsh (6) referred to a notice of the occurrence of a parasitic fly as follows:

> But in the year 1863, as we learn from Isaac Hicks, of Long Island, a deliverer appeared in the form of a small shining black parasitic fly, probably belonging either to the Chalcis or to the Proctotrupes family. Whether this fly lays its eggs in the eggs of the asparagus beetle or in the larva of that insect does not seem to be at present clearly ascertained; but if the accounts that we have received of it be correct it must do either one or the other. In the former case the larva that hatches out from the parasitic egg will consume the egg of the asparagus beetle and entirely prevent it from hatching; in the latter case it will destroy the larva before it has time to pass into the perfect state. The result in either event will be equally destructive to the bug and beneficial to the gardener.

Later, in 1882, Lintner (4) made notes on the same species, referring to the publication in the American Entomologist just quoted. Again in 1893 (5) he called attention to a parasite, stating that it was undescribed and that it might have disappeared before it could receive scientific attention, because nothing seemed to be known of it at that time.

From the descriptions given it seems almost certain that the parasite mentioned by Riley and Walsh and *Tetrastichus asparagi* Cwfd. are the same. If so, it is hard to explain why this insect, which was reported in considerable numbers in 1863, should have escaped further observation until 1909.

[1] Reference is made by number to "Literature cited," p. 312.

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K—19

So far as known by the writer, the life history of this parasite had not been studied, and as it appeared in considerable numbers during the season of 1912 near Riverhead, N. Y., where the writer was stationed, a study of its life history and habits was undertaken. During the fall of 1912 Mr. H. M. Russell, of the Bureau of Entomology, also stationed at Riverhead, and the writer published a short article (7) on this parasite, based principally on observations made during that season.

DESCRIPTION OF THE PARASITE

The adult (Pl. XLIX, fig. 1) of *T. asparagi* was described by Mr. Crawford as follows:

THE ADULT

Female.—Length 2 mm. Belongs to the group of *T. hylotomae* Ashm.; dark blue green; face finely reticulate and with scattered punctures; antennæ with one ring joint; joints 1–3 of flagellum almost equal, the first slightly longer and about as long as the pedicel; third flagellar joint hardly longer than wide and about as long as the first joint of club; mesothorax finely longitudinally rugulose, the median furrow failing anteriorly; middle lobe of mesonotum with a single indistinct row of punctures on each side; metathorax roughened, median and lateral carinæ strong; metathorax at median carina much longer than postscutellum; coxæ, trochanters, and femora, except apices, green, the rest of the legs reddish testaceous.

Amherst, Mass., reared from eggs of *Crioceris asparagi* by Dr. H. T. Fernald.

Type.—Cat. No. 12676, U. S. Nat. Mus.

This species is very closely related to *T. hylotomae*, but has shorter antennæ. In the female of *T. hylotomae* the third joint of the flagellum is twice as long as wide, and distinctly longer than the first joint of the club, the first joint of the flagellum is one and one-half times as long as the pedicel; the median furrow of mesonotum is distinct to the anterior margin.

The following descriptions of the egg, larva, and pupa of *Tetrastichus asparagi* are by the author.

THE EGG

The egg (Pl. XLIX, fig. 2) is reniform in shape, with one end more slender than the other, about 0.24 mm. long and 0.08 mm. wide, semitransparent, and is of a milky color, with a granular appearance within. While the eggs may be laid singly, in a number of cases they were found side by side in pairs (Pl. XLIX, fig. 3).

THE LARVA

The mature larva (Pl. XLIX, fig. 4) is from 2 to 2.5 mm. long and about 1 mm. wide. It is white, with the alimentary canal appearing greenish; ovate; widest near the head, which is contracted and bent under the body. The surface is smooth and devoid of hairs. There are no legs, and the larva seems incapable of motion, except to move the end of the abdomen when disturbed.

THE PUPA

The pupa (Pl. XLIX, fig. 5) is from 2 to 2.5 mm. long and from 1 to 1.2 mm. wide. It is yellowish white; convex dorsally, with the head somewhat bent under and inconspicuous wing pads folded along the side (Pl. XLIX, fig. 6). The antennæ and legs are folded under ventrally. The head, thorax, and abdomen are distinctly differentiated from one another; the abdomen tapers posteriorly.

DISTRIBUTION OF THE PARASITE

This insect has been recorded from Amherst and Concord, Mass., by Dr. H. T. Fernald; by Mr. D. E. Fink, of the Bureau of Entomology, from Ithaca, N. Y.; and the writer has observed it in considerable numbers on Long Island. During June and July, 1911, specimens of this insect were liberated at Jessup, Riverdale, and Rives, Md., but at the present time it has not been retaken in these places. In all probability, however, it is present in many localities in the northeastern part of the United States, other than those mentioned.

OCCURRENCE ON LONG ISLAND

On June 10, 1912, the author first observed this parasite at Aquebogue, Long Island, while examining an asparagus field. Large numbers of this insect, together with the asparagus beetle (*Crioceris asparagi*) and its eggs, were found on check rows of asparagus which had been left in the field to attract the beetles from the main crop. At times as many as six or seven parasites were to be seen on a single stalk. These check rows had not been set aside as a trap for the beetles until a short time before, and as a consequence the asparagus was not over a foot high and had not branched out. As a result, the beetle eggs were confined to a limited area, and it was fairly easy to follow the actions of the parasites.

FEEDING OF PARASITES

This insect is an energetic feeder on its host's eggs and is evidently as useful in checking the host in this way as by its parasitic development, if not more so.

When a careful examination of the beetle eggs on stalks of asparagus was made, many were found that had collapsed and withered, and it was quite evident that they would never hatch. On some asparagus stalks the only viable eggs appeared to be those recently deposited. The cause of this collapsed condition of the eggs was soon apparent. A female adult parasite under observation approached an egg, and, after carefully examining it with her antennæ, climbed upon it, inserted her ovipositor, and worked it up and down with a pumping motion. This motion was continued for varying lengths of time, from a few seconds to three or four minutes, after which she withdrew the ovipositor, backed down from the egg, and, applying her mouthparts to the puncture, sucked up the egg contents.

She usually fed from the egg until the shell collapsed. At times it was necessary for her to manipulate the ovipositor in the egg four or five times before the contents were sufficiently loosened to permit their extraction. This feeding of the parasite was so extensive that of 2,097 eggs counted on 28 stalks of asparagus, 1,495, or 71.29 per cent, had been destroyed.

LABORATORY EXPERIMENTS

Several of the adult parasites, captured and confined with the eggs of the asparagus beetle in the laboratory, were noted shortly afterward, ovipositing and feeding on the beetle eggs. A few days later all eggs of *Crioceris asparagi* which had not been eaten by the parasites had hatched. At the time this could not be accounted for, since this insect had previously been considered an egg parasite and many of the eggs which hatched were known to have been subjects of oviposition. None of the young beetle larvæ that hatched from these eggs were carried through to maturity. The cause of their death appeared to be a lack of proper food.

On the first day that the parasites were observed in the field, Mr. Russell collected nearly mature larvæ of the asparagus beetle from volunteer asparagus plants in a field which had been planted to asparagus at some prior date. The larvæ were taken to the laboratory and placed in rearing cages that they might form their cells. A few days later, while the cocoons were being examined, six small whitish larvæ were found in one cocoon. Some of these larvæ at a later date pupated, but died before the adult stage was reached, so there was no certainty that these were the larvæ of *T. asparagi*.

About July 10 the writer collected asparagus-beetle larvæ from a field in which parasites had been previously noted in abundance and, bringing them into the laboratory, supplied them with food and confined them in vials without earth.

Upon examining the vials on July 24 it was seen that five beetles had emerged and in one vial there were three small pupæ. In another vial was a small whitish larva similar to those which Mr. Russell had previously taken from a beetle cell.

The three pupæ were placed in a separate vial, and on July 30 and 31 they emerged as adults, which were later identified by Mr. Crawford as *T. asparagi*.

On June 20 the writer dissected the egg of *T. asparagi* from the asparagus-beetle egg, and the peculiar life history of this parasite was at length established.

For nearly two weeks after the parasites were first observed in the field they were to be found in considerable numbers, after which they suddenly disappeared; by June 24 none could be found. During the latter part of July they again made their appearance in the field but were much harder to locate, since the entire asparagus field by this time had been allowed to grow, and, in consequence, the parasites were scattered over a much larger area than before.

On August 5 the first parasites of a second generation were captured in the field and brought into the laboratory. They were confined in large vials and each day were given a fresh supply of beetle eggs, which were treated as noted before. On hatching, the beetle larvæ were removed to

another vial and supplied daily with fresh food. At maturity they went into the soil in the bottom of the vial, and in due time either adult beetles or the parasites issued from the soil.

METHODS USED FOR REARING THE PARASITES IN CONFINEMENT

The adults of the parasite *T. asparagi* were captured in the field and confined in the laboratory in vials, measuring 100 mm. in length and 28 mm. in diameter. The ends of the vials were covered with cheesecloth, as better results were obtained when this was used than when the vials were stopped with cotton plugs. Each day a supply of fresh asparagus-beetle eggs was collected in the field, brought into the laboratory, and a certain number placed in a vial with each parasite. The eggs remained with the parasites for 24 hours, when they were removed, the number parasitized and the number eaten by each parasite being recorded and the twigs bearing the eggs placed in moist sand, so that the eggs might hatch.

As soon as the young beetle larvæ hatched, they were confined in vials about one-third full of moist earth, and supplied with fresh food each day. As soon as the beetle larvæ were full grown, they were allowed to go into the soil in the vial and pupate. In several cases the pupal cells were formed near the glass, and it was possible to observe the naked parasitic larvæ in the cell after they had completely consumed their host.

LENGTH OF LIFE OF ADULTS IN CONFINEMENT

Tables I, II, and III show the length of life of adults collected in the field and those reared in the laboratory, and the number of eggs parasitized and eaten by each parasite.

TABLE I.—*Length of life of the parasite Tetrastichus asparagi, reared at the Riverhead, N. Y., laboratory in 1912*

Date of emergence.	Date of death.	Number of eggs parasitized.	Number of eggs eaten.	Length of life.[a]	Average number of eggs parasitized daily.	Average number of eggs eaten daily.
				Days.		
Sept. 3	Sept. 9	18	26	[b]6	3	4.33
3	9	16	30	6	2.66	5
3	9	14	32	6	2.33	5.33
3	10	11	25	7	1.57	3.57
3	8	15	20	[c]5	3	4
3	10	11	22	7	1.57	3.14
12	17	0	0	5	.....	.....
12	17	0	0	5	.....	.....
12	18	10	10	6	1.66	1.66
12	22	19	27	10	1.99	2.7
12	18	0	1	6	.....	.....
12	Oct. 7	40	61	[d]25	1.81	2.77

[a] The average length of life was 7.83 days; the maximum, 25 days.
[b] All died on Sept. 9, when they were left from morning until afternoon without any eggs in the vial.
[c] This parasite was accidentally killed.
[d] During the last 3 days no asparagus-beetle eggs could be obtained.

TABLE II.—*Length of life of adult Tetrastichus asparagi at the Riverhead, N. Y., laboratory in 1912*

Date of capture.	Date of death.	Number of eggs parasitized.	Number of eggs eaten.	Length of life.[a]	Average number of eggs parasitized daily.	Average number of eggs eaten daily.
				Days.		
Aug. 5	Aug. 11	0	7	[b]6	0	3.5
5	12	9	11	[c]7	3	3.66
7	11	13	22	4	3.25	5.5
7	14	22	44	7	3.14	6.28
8	19	41	50	11	3.72	4.54
8	19	17	51	11	1.54	[d]4.63
12	14	0	6	2	0	3
12	17	16	28	5	3.2	5.6
12	26	7	37	14	.5	[d]2.64
17	21	0	9	4	0	2.25

[a] The average length of life was 7.1 days; the maximum, 14 days.
[b] Records for the last 2 days only.
[c] Records for the last 3 days only.
[d] Several eggs hatched in the parasite vials.

TABLE III.—*Life cycle of Tetrastichus asparagi at the Riverhead, N. Y., laboratory in 1912*

Date of oviposition.	Date of emergence.	Number emerged.	Length of stages.	Remarks.
			Days.	
Aug. 6	Aug. 30	6	24	Was removed from the soil.
6	30	6	24	
8	30	[a]2	.....	
8	Sept. 2	5	25	
8	3	6	26	
9	2	7	24	
9	5	5	27	
11	5	4	25	
11	6	6	26	
6	6	2	31	
6	7	2	.....	Dead adults taken from top of the soil.
9	7	5	29	
6	11	[b]7	.....	The seven adults were dead in the soil.
15	12	6	28	
15	15	6	31	
15	16	[c]2	.....	Were taken from dirt near top of vial.
17	16	7	30	
6	21	.....	.....	Four larvæ and nine dead adults taken from soil in vial.
8	25	.....	.....	Four larvæ dug out of soil in vial.
Sept. 5	Oct. 11	7	36	Second generation.

[a] Pupa. [b] Adults. [c] Dead.

SEXES

During the time that this parasite has been under observation only females have been found, both among the adults collected in the field and among those reared in the laboratory, and reproduction has been parthenogenetic, so far as has been observed. Of two generations that have been reared in the laboratory, no males have appeared.

Parasites which were separated as soon as they emerged, and confined with asparagus-beetle eggs, immediately commenced feeding and ovipositing and another generation was reared from the parasitized eggs. In one case where six parasites emerged in one vial and were immediately separated, each being given beetle eggs, five of the six were observed to feed on an egg within 15 minutes after they were placed with the eggs, while the other one was observed to oviposit first. From this it would seem that, as a rule, the parasite after emerging feeds on a few eggs before beginning to oviposit. These five adults were observed to oviposit later in the day.

NUMBER OF GENERATIONS

Apparently this insect produces three generations a year on Long Island, for it was very abundant early in June, when it disappeared, to be found again in July, after which time two generations were reared in the laboratory. However, indications are that the third generation, in the fall, is only a partial one.

In one case three beetle eggs were found to be parasitized on August 9, and on August 11 the beetle larvæ hatched and were given food. On August 17 they went into the ground to pupate, and on September 7 five adult parasites emerged. On January 3, 1913, when the soil in the vial was taken out and examined, it was found that one of the two cells which were still in the ground held five dead adult parasites. In the other cell there were five parasitic pupæ. These pupæ, being confined in a warm room, immediately began to change and on January 8 emerged as adults. In another case, from beetle larvæ hatched on August 11 from eggs that had been confined with one parasite, 10 parasites emerged on September 5 and 6. As no more issued from this vial, the soil in it was taken out and examined February 3, 1913. In one cell were found five parasitic larvæ. These larvæ pupated February 7 and on February 17 were emerging as adults. From these facts it would appear that the last generation was but a partial one. The fact that in the laboratory experiments representatives of the third generation emerged in one vial only, whereas on examining the soil in some of the vials during January a number of parasitic larvæ were found, would indicate that the third generation might be the exception instead of the rule.

HIBERNATION

During the latter part of January and the first of February the soil in several vials was examined in order that the stage in which this insect passed the winter might be ascertained. Seven cells containing parasites were found, in six of which they were in the larval stage, while in the seventh they had passed to the pupal stage. This would indicate that the insect hibernates as a full-grown larva in the cell of its host in the ground.

NUMBER OF PARASITES EMERGING FROM SINGLE HOST LARVA

In dissecting eggs of the host, from 1 to 5 eggs of the parasite were found, and in the rearing experiments undertaken in the laboratory from 1 to 10 larvæ of the parasite have been found in a single beetle cell. However, the usual number of parasites that issued from one host larva was from 5 to 7. In two cases only were more than 7 parasites found in a single host and in 1 of these 10 and in the other 9 were found. There was one case where only 1 parasite was found in the host, but as mites had destroyed several cells in this vial and were also in this cell, it seems strongly probable that they had destroyed some of the parasites in this particular cell.

ONLY HOST

The asparagus-beetle egg parasite has been observed attacking only the eggs of the common asparagus beetle (*Crioceris asparagi*). In the laboratory it has been confined with the eggs and young larvæ of the potato beetle, and with the eggs of the elm leaf beetle, but it paid no attention to them.

PUPATION AND THE PUPAL PERIOD

The pupa when first formed is yellowish white throughout. Shortly the eyes become reddish and the mandibles darken. In from two to three days the eyes are bright red and the ocelli are also visible and are of the same color. Next, the head and thorax begin to turn black and this continues on through the abdomen, until just before emergence the whole pupa appears black.

Parasitic larvæ which were seen in a cell on August 20 emerged as adults on August 30. Another brood first seen on August 26 on September 7 emerged as adults.

In a vial in which parasite larvæ were seen on August 20, adult parasites emerged August 30.

Parasitic larvæ which were taken from the soil on January 25, 1913, and kept in a warm room pupated on January 30 and the adults emerged on February 8.

Another lot of larvæ taken from the soil on February 3 pupated on February 7 and emerged as adults on February 17. According to these data, the pupal period lasts from 7 to 11 days.

OVIPOSITION

The process of oviposition is in some respects different from that of feeding. The parasite crawls slowly over the plant with the antennæ held down in front of the face and kept in constant vibration. When a beetle egg is encountered it is carefully examined with the antennæ and, if satisfactory, the parasite crawls upon it and inserts the ovipositor. The ovipositor remains in the egg for a few minutes, without the pul-

sations of the abdomen noticed when the parasite feeds on an egg. It is then withdrawn, and the parasite leaves the egg. In one or two instances it appeared that the parasite after ovipositing in an egg returned to it and repeated the act of oviposition.

In another case a parasite observed in the act of oviposition was approached by a second individual which, climbing up on the opposite side of the egg, began to work the ovipositer up and down in the egg in preparation for feeding. Each was aware of the other's presence, but paid no observable attention to the other.

Table IV gives the time required for oviposition and feeding for a few individuals.

TABLE IV.—*Length of oviposition and of feeding of Tetrastichus asparagi at the Riverhead, N. Y., laboratory in 1912*

Length of oviposition.						Length of feeding.		
Parasite No.	Minutes.	Seconds.	Parasite No.	Minutes.	Seconds.	Parasite No.	Minutes.	Seconds.
1	.....	33	12	1	[a]42	1	23	30
2	2	5	13	2	15	2	8	30
3	3	25	14	3	32	3	11	35
4	2	15	15	2	25	4	9	27
5	1	.....	16	3	30	5	9	.....
6	2	30	17	4	43	6	6	.....
7	.....	40	18	.....	25			
8	.....	[a]55	19	4	.....			
9	2	[a]25	20	4	12			
10	1	10	21	3	10			
11	.....	[a]36	22	3	.....			

[a] Same eggs.

EVIDENCE OF PARASITISM

By means of a hand lens the beetle eggs which had been parasitized were readily distinguishable. Where the ovipositor punctured the egg, a small circular area appeared which projected slightly from the rest of the eggshell and which had a shiny appearance, caused by the small amount of the contents of the egg which had oozed from the puncture.

Beetle larvæ hatching from parasitized eggs appeared normal and continued to feed and grow until maturity. When matured, they went into the ground and prepared their cells for pupation, but here their activities stopped, and in a few days the cell was occupied by the parasitic larvæ, all that remained of the beetle larva being the empty skin.

IMPORTANCE OF THIS PARASITE

The asparagus-beetle egg parasite is of considerable importance, as it not only attacks the host during its parasitic development but is also beneficial in destroying its host's eggs through feeding; in fact, it ap-

pears to be of greater value as an egg destroyer than as a parasite developing within the host.

Mr. C. W. Prescott, of Concord, Mass., recently wrote that on May 23, 1909, he had noticed the parasite in the field feeding on the host eggs, and that on the day of writing he had attempted to find "slugs" or larvæ, but could find neither slug nor egg except those absolutely dry, in a field of 5 acres.

During the season of 1912, the field of asparagus at Aquebogue, N. Y., where this insect was found, received no treatment for the beetles, yet these were so scarce that no damage resulted. Previous to this, according to the owner, the field had been sprayed each year to prevent serious injury.

Without doubt this parasite was to a large degree responsible for the scarcity of the asparagus beetles. Certain other factors may have assisted, but there is little doubt that the parasites were the most important factors in preventing damage.

SUMMARY

Previous to the time that *Tetrastichus asparagi* was believed to be an egg parasite, its life history had never been worked out. As the parasite had been observed ovipositing in the host egg, it was supposed that it developed in the egg. During the investigation of the life history it was discovered that this insect presented one of those peculiar instances where oviposition in the host's eggs and retarded development of the parasite permitted the host to develop. In this case the following takes place:

The parasite deposits her eggs in the egg of the asparagus beetle; the beetle egg hatches; its larva feeds to maturity and entering the soil forms a pupal cell, but does not pupate. The parasites have by this time totally consumed the larva and emerge from it into the cell the larva has constructed, where they pupate and later emerge as adults.

Since the parasitic larva passes the winter in the soil in the pupal cell of its host, it would appear that the parasite might easily be transported from one place to another in the soil which might surround a shipment of asparagus roots.

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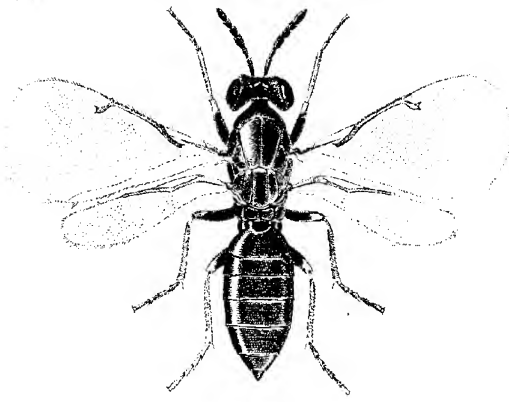
PLATE XLIX

Tetrastichus asparagi:

Fig. 1.—Female adult. Highly enlarged. Original.
Fig. 2.—Egg, laid singly. Highly enlarged. Original.
Fig. 3.—Eggs, laid in pairs. Highly enlarged. Original.
Fig. 4.—Larva. Highly enlarged. Original.
Fig. 5.—Pupa, ventral view. Enlarged. Original.
Fig. 6.—Pupa, side view showing inconspicuous wing pads. Enlarged. Original.



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INHERITANCE OF CERTAIN CHARACTERS OF GRAPES

By U. P. HEDRICK, *Horticulturist*, and R. D. ANTHONY, *Associate Horticulturist*, *New York Agricultural Experiment Station, Geneva, N. Y.*

INTRODUCTION

The breeding of grapes (*Vitis* spp.) was begun in the Horticultural Department of the New York Agricultural Experiment Station about 25 years ago and has been continued throughout this time as a horticultural problem. Nearly 10,000 seedlings have been grown, and of these about 6,000 have fruited. This work was begun about 1885 by Prof. E. S. Goff, who at first grew seedlings and plants from seeds open to cross-pollination. Later he crossed a number of varieties. In 1891 Prof. S. A. Beach became the Station horticulturist, and besides seeking to obtain new varieties he made studies of self-sterile varieties, studied the correlation between the size of seeds and vigor of plants, and did considerable hybridizing. In 1905 the senior author took charge of the work in horticulture at the Station. Mendel's work had just been discovered, and plant breeding was undergoing a stimulus from it. The work with grapes was therefore replanned and extensively added to with a view to studying problems of inheritance. This work has been continued and increased from year to year. Several assistants and associates have spent much of their time working with grapes at this Station. Mr. N. O. Booth worked with grapes from 1901 to 1908; Mr. M. J. Dorsey from 1907 to 1910; Mr. Richard Wellington from 1906 to 1913; Mr. R. D. Anthony, the junior author, began work at this Station in the summer of 1913, and has devoted most of his time to the grape work since then. Upon him has fallen the task of presenting the data in this paper. It is the purpose of this paper to discuss certain results of this work.

AIMS, METHODS, AND MATERIALS

During this quarter of a century, experience and a better understanding of the principles of breeding have modified many of the methods and changed considerably the nature of the data which are now taken.

The ultimate aim in this work is, of course, the production of improved horticultural varieties. Through the early days, when breeding laws and methods were less understood than now, there was a tendency to make this the immediate as well as the ultimate aim. The fact that the first 20 years of grape breeding produced but one variety worthy a name served to confirm the conviction that this goal would be reached quicker by for-

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getting it for the time being and bending every effort to the discovery of how grape characters are transmitted.

The work is now proceeding mainly along two lines: (1) The determination of the breeding potentialities of a considerable number of varieties of grapes, especially with the view of finding unit characters; and (2) a review of all the New York Experiment Station breeding data on this fruit, to study and interpret breeding phenomena, accompanying this review with the making of the crosses necessary to throw further light upon doubtful points.

The results secured in testing the breeding possibilities of grape varieties, which will be discussed later, have made it seem desirable to extend this study to all the varieties which show any promise. For this reason nearly 200 different kinds have been used as pollen parents and nearly 100 as maternal parents in this work.

Frequently during the early days of the work seedlings which seemed to lack vigor in the nursery were discarded, instead of being planted in the test vineyards. Though this undoubtedly removed many unpromising seedlings, it seriously decreased the number which fruited, and made the interpretation of results difficult and uncertain. At best the number of seedlings that lived of each cross was smaller than could be desired, and, when this number was still further decreased by selection in the nursery or by untoward circumstances, much of the value of the work from a breeding standpoint was lost.

Another change of method which bids fair to be exceedingly important has been the use of varieties of *Vitis vinifera* in breeding. Every indication points to the desirability of the addition of some of the blood of the European grape to our native sorts. Although we are working primarily to determine breeding laws, there is usually a wide choice of varieties which answer our purpose, and with the growing of nearly 100 varieties of this species on the New York Station grounds we have been able to use several as parents. There are now several hundred hybrids containing *V. vinifera* blood growing on the Station grounds, and these will be increased by many hundreds during the following years.

The methods used in the actual work of crossing are similar to those of most breeders. The female blossoms are emasculated before the calyx cap splits off and are then bagged; the male blossoms are also bagged before the calyx splits. When the pollen is ripe, the bagged male cluster is usually cut from the vine and all or part of it brushed over the emasculated female. Usually some of the male cluster is inclosed in the bag, which is again put over the female after pollination. In a few cases, where the periods of blossoming of two varieties are too widely separated, it has been necessary to save pollen in clean glass jars. It is customary to dip the forceps used in pollination into alcohol with each new variety.

Certain results secured in the summer of 1914 seem to indicate that this method is perhaps open to criticism. While emasculating clusters of the Janesville variety it was found that, although the cap had not split, the pollen in the anthers seemed to be mature, and, as the anthers were ruptured during the emasculation, there was a possibility of self-fertilization taking place. Several clusters were emasculated and bagged without being pollinated. These set nearly the full quota of berries with seeds that have every appearance of being viable. With two other varieties, clusters emasculated and not pollinated matured a few plump seeds, though the clusters were much below normal. A somewhat similar instance is reported by Beach (1),[1] the variety being the Mills. This point deserves careful study, for if it is found that serious danger of self-pollination exists before the calyx cap splits it will be necessary to change the method—at least to the extent of emasculating the clusters several days before the cap is ready to come off.

All data regarding size and shape have been recorded in comparative terms, instead of the actual measurements being taken. With a limited number of observers and thousands of seedlings of the various fruits to be studied each year, it was a physical impossibility to take measurements and would not have increased the value of the records to any extent from a horticultural point of view, though it would, of course, have furnished interesting material for a statistical study. The value of data reported in comparative terms depends upon the accuracy of the recorder. The work with the grape has always been done by members of the scientific staff, and the observations have usually been checked during several seasons.

GENERAL RESULTS OF THE STUDY OF VARIETIES OF GRAPES

One of the surprises in the study of varieties of grapes was the failure of many of our commercial sorts to transmit desirable qualities to their progeny. Seedlings of Concord, Niagara, Worden, Delaware, and Catawba grapes have so far proved only disappointments. The best results have been secured from such little-grown varieties as the Ross, Collier, Mills, Jefferson, Diamond, and Winchell. This has made it seem desirable to test all varieties that show any promise. The first step, then, was to secure as many varieties as possible which were of any value and which could be grown in northeastern United States. More than 400 such varieties have fruited in the Station's vineyards and have been described. About 200 of these have been used to a greater or less extent in the breeding work.

As an aid in studying the breeding possibilities of grape varieties the Station has grown nearly 3,000 selfed, or pure, seedlings, using as parents most of those varieties which have entered into the crosses that have been

[1] Reference is made by number to "Literature cited" p. 329-330.

made. These seedlings have thrown much light upon the inheritance of various factors, but they have been so uniformly lacking in vigor as to lead to the belief that only through crossbreeding can we hope to produce improved varieties.

INHERITANCE OF CHARACTERS

The grape characters discussed in the following pages are those for which sufficient data are available to make such a discussion of value.

SELF-STERILITY

On the basis of flower type, grapes may be divided into three classes: (1) True hermaphrodites; (2) hermaphrodites functioning as females, owing to completely or partially abortive pollen; and (3) pure males with the pistil absent or rudimentary. Among these classes there are two types of stamens: Those with upright filaments and those in which the filaments bend backward and downward soon after the calyx cap falls off. According to Dorsey (5), this is due to the cells of the outer surface of the reflexed stamens being smaller and having thinner walls.

So far as observed at the New York Station, all pure males have upright stamens. Among the two classes of females Beach (1) found that only those varieties with upright stamens were capable of producing marketable clusters of fruit when self-fertilized. At the same time he reported nine varieties with upright stamens to be self-sterile. Since Beach published his report further work at this Station with three of these nine varieties has proved them self-fertile, and it is probably safe to say that all varieties with upright stamens are self-fertile, though in varying degrees. Reflexed stamens are always correlated with complete or nearly complete self-sterility. Reimer and Detjen (6) found this last conclusion to hold also with *Vitis rotundifolia*, a species not studied at this Station.

The cause of self-sterility in varieties with reflexed stamens seems to be a lack of viability in all or a large part of the pollen of such varieties. Booth (2) found that such pollen was quite irregular in form and would not germinate in sugar solutions. Reimer and Detjen (6) state "the pollen of all the present cultivated [female] varieties [of *V. rotundifolia*] is worthless." Recently Dorsey (4) has ascribed the cause of this self-sterility to a degeneration in the generative nucleus. While this impotency may be absolute in many of the varieties, in some at least it is only relative. Frequently viable pollen will be found mixed with the usual misshapen, abortive pollen of the self-sterile varieties, and nearly a hundred pure seedlings of the varieties which Beach (1) reported as totally self-sterile have been grown in the Geneva Station vineyard. The degree of sterility seems to depend to some extent upon the condition of the vine due to environmental factors.

From a practical standpoint it is undesirable to grow self-sterile varieties. They will not succeed in the large blocks of the commercial plantation, nor are they always properly fertilized in the small home vineyard. Can we, then, in our grape breeding eliminate self-sterility? Letting U stand for upright stamens and R for reflex, the following table gives the results of our crosses:

U×U[1] =180U+ 47R	R×R = 16U+16R	R×U =207U+206R
U selfed=691U+152R	R selfed= 94U+73R	Ratio 1U:1R
Total...871U+199R	Total...110U+89R	U×R=?
Ratio 4.3U:1R	Ratio 1.2U:1R	

Of the varieties whose pure seedlings have entered into the ratio of 4.3 U to 1 R, only two, involving 18 seedlings, have given simply upright stamens; consequently it may safely be said that no variety has proved pure for upright stamens. In the remaining crosses of this class the ratios have ranged from 1 U to 2 R up to 10 U to 1 R, with the greatest frequency at 2 U to 1 R. The results with the crossed seedlings are practically the same. Over a thousand seedlings from crosses of one type would be expected to give some rather definite results; yet these results are anything but definite, and apparently no conclusions can be drawn from them except that the varieties are not homozygous for uprightness of stamens.

The ratio of practically 1 to 1 in crosses of varieties with reflexed stamens is perhaps best accounted for by the supposition that the gametic composition of pollen and ovules is not alike. The ratio of 1 to 1 in crosses of reflexed stamens by upright may be covered by the same assumption. It should be noted that the pollen of the upright varieties produces the same ratio as that of the reflexed varieties when both are used on ovules of the reflexed kinds.

Upright varieties have been crossed many times with reflexed sorts and several hundred seedlings should have resulted from these, yet only one plant has survived the vicissitudes of the seed bed and nursery to be planted in the test vineyards. In the last five years 50 crosses have yielded 600 seeds; yet from these there are now in the nurseries but 25 living seedlings. Many of the pollen parents used in these crosses were the same as those used in the cross R×R. In the two crosses R×R and R×U, the pollen from upright and reflexed varieties produced the same results; but comparing this last case, U×R, with the first one, U×U, we see that the pollen of the upright and reflexed varieties has produced quite different results when used on upright sorts. Why this should be is not apparent.

At present there does not seem to be any way of eliminating reflexed stamens, but we can at least decrease the proportion by using for breeding only varieties with upright stamens.

[1] The pollen parent is always placed last.

INHERITANCE OF SEX

Among more than 6,000 seedlings which have flowered in the New York Experiment Station vineyards, less than 100 pure male vines have been found. Of these there are complete breeding records for 62 vines, 51 of which came from crosses in which the pollen parent was a pure male, leaving 11 males recorded as produced by pollen from hermaphrodite plants. Of these, 5 were pure seedlings from one parent, the other 6 from 5 crosses. These 6 were probably hermaphrodites erroneously recorded as males, an error very easy to make when the pistil has a short style and one which has been made several times and corrected by subsequent observations. The parent yielding the 5 males was discarded shortly after being used in breeding, and our records are meager. It was probably an intermediate recorded as a hermaphrodite. Such an intermediate, having both male and hermaphrodite blossoms, is under observation in one of the Station vineyards, and its pollen seems to behave as the pollen of a pure male; in other words, it is reasonable to assume that, excluding these intermediate forms, pollen from hermaphrodite plants will not produce pure males.

The results obtained from pure males as pollen parents are:

Hermaphrodite female × pure male =
56 hermaphrodites + 51 pure males

Following the assumptions usual to such cases, the hermaphrodite would be considered a sex heterozygote and the male a sex homozygote. Yet selfed hermaphrodites yield only hermaphrodites. These results are similar to those obtained at this Station from selfed hermaphrodite strawberries, but differ from Shull's results (7) with species of Lychnis, where the hermaphrodite gave both females and hermaphrodites. This condition might be covered by the assumption that the hermaphrodite is a female in which the addition of a single dose of maleness has caused the production of male organs, the ovules keeping the composition ♀ ♀ and the pollen becoming ♂ ♀:

Hermaphrodite × hermaphrodite = ♀ ♀ × ♂ ♀ = 2 ♂ ♀ + 2 ♀ ♀

Since we have no pure females, we must assume that some condition prevents the formation of individuals with the composition ♀ ♀; therefore, the above cross gives only hermaphrodites. Of course, if we do not attempt to assume the method of origin of the hermaphrodite, the case may be covered by considering the hermaphrodites pure for this character, while the males would be heterozygous:

$$\text{♀}\,\text{♀} \times \text{♀}\,\text{♀} = \text{♀}\,\text{♀}$$
$$\text{♀}\,\text{♀} \times \text{♀}\,\text{♂} = \text{♀}\,\text{♀} + \text{♀}\,\text{♂}$$

COLOR OF SKIN

Colors of grapes are not sharply differentiated, grading from white through many shades of red and purple to black. Because of this wide range, the problem of finding varieties which are pure for certain colors

has been greatly complicated, yet until we are able to find such pure colors and to study their various combinations our knowledge of the color composition of many varieties will probably be only conjectural.

The thousands of seedlings which have been fruited have made possible the formulation of but two general laws: (1) White is a pure color; and (2) white is recessive to both black and red. White, yellow, green, and amber are all considered under the one term and are regarded as being the absence of red and black.

No black variety that has been tested to an extent sufficient to make the results at all conclusive has proved pure for blackness. Some have a factor for red; others seem to contain only black and its absence, white, while still others have both white and red progeny.

In order to simplify the study of red varieties, it has seemed best to divide the seedlings into two shades, the light or medium reds and those ranging from dark red to purple. Those in the second classification are probably red plus either an intensifying factor or various amounts of black. Such a division is somewhat arbitrary and some colors are difficult to place, but, in general, it is a helpful arrangement. Table I gives the result of combining similar colors and shows that the results obtained from crosses of red varieties are as diverse as those from the black. The table includes mainly pure seedlings.

TABLE I.—*Results of crossing grapes of similar colors*

Colors of parental types.	Colors of seedlings.			
	Black	Purple to dark red.	Medium to light red.	White.
White × white				166
Light red × light red	8	6	13	8
Dark red × dark red	38	43	45	42
Black × black	407	49	13	54

Table II illustrates the variation in color composition among most of the varieties given above in the cross black × black and shows the number of varieties which fall in similar groups. From this table it will be seen that 15 black varieties have given only black seedlings, but the number of seedlings is not large enough to be conclusive.

TABLE II.—*Color groups of pure seedlings of black varieties of grapes*

Number of parental varieties.	Colors of seedlings.		
	Black.	Red.	White.
6	52	16	29
6	128	31	.....
10	71	.....	25
15	132	.....	.....

It is interesting to note that the parents with only black and white seedlings produce these colors in the ratio of 3 to 1 and that the seedlings of the varieties yielding only black and red are reasonably close to this same ratio.

The results when different colors are combined are given in Table III. The range of color in the seedlings again emphasizes the necessity of knowing more of the color composition of a variety than can be determined from its appearance.

TABLE III.—*Results of crossing grapes of different colors*

Color combinations of parents.[a]	Colors of seedlings.			
	Black.	Purple to dark red.	Medium to light red.	White.
White × dark red [b]	5	44	14	50
White × black	41	3	3	12
Black × dark red	100	52	40	32

[a] Light-red varieties were not used to an extent sufficient to make the results of value.
[b] The reciprocal cross is included in each case.

It would take up altogether too much space to report upon the color of the progeny of all the varieties studied, but a few of the more common ones are given in Table IV, in order to show the wide variation in different varieties of similar color.

TABLE IV.—*Variation in color of pure seedlings of certain varieties of grapes*

Parent.		Pure seedlings.			
Name of variety.	Color.	Black.	Purple to dark red.	Medium to light red.	White.
Agawam	Purple red	1	2	.....	2
Brighton	Dark red	6	5	9	7
Catawba	Purple red	2	4	3	4
Champion	Black	13	1	1	2
Clinton	Black	15	.....	.....	7
Concord	Black	40	6	.....	12
Essex	Purple black to black	4	2	3	.....
Hartford	Black	4	.....	1	3
Hercules	Black	3	1	1	10
Isabella	Black	8	.....	.....	1
Merrimac	Black	9	3	0	.....
Nectar	Black	4	5	2	.....
Ozark	Black	16	.....	.....	.....
Pearl	White	.....	.....	.....	15
Regal	Dark red	.....	.....	15	5
Worden	Black	4	3	.....	1
Wyoming	Dark red	1	4	2	3

In the ultimate solution of the problems of color inheritance we shall probably be aided in no small degree by those who are studying the subject from the standpoint of the chemistry of the various colors; thus,

Wheldale (8) has isolated two anthocyanins from species of Antirrhinum which produce different shades of red and three flavones for ivory, yellow, and white. Some work has already been done along this line with the grape. Dezani (3) has found two chromogenic substances in white grapes, and several have reported work on the coloring matter of red grapes, but apparently the results are as yet too indefinite to be of much value to the breeder.

INHERITANCE OF QUALITY

At first thought it would seem useless to attempt a study of such an elusive and composite character as quality the interpretation of which depends so much upon the tastes of the observers; yet in the final analysis it is this character which very largely determines whether a seedling is worth saving or must go to the brush pile, and any addition to our knowledge of its inheritance is worth the effort.

Table V shows the rating of the progeny of various parental combinations which run the gamut of quality. Most noticeable is the very low percentage of seedlings whose quality is good or above good even when parents of the highest quality were used. When we consider the ancestral history of these seedlings, these results are not surprising or discouraging. Our American grapes, except for the *V. vinifera* hybrids, are but a step removed from the wild, only a few possessing sufficient quality to make them stand out from the many thousands too poor to be eaten with relish. In breeding from these we are breeding from the topmost point of the species and the effect of the several hundred poor kinds in the immediate ancestry is to pull the seedlings down toward the "level of mediocrity."

TABLE V.—*Inheritance of quality in grapes*

Parental types.	Types of progeny.											
	Best.	Very good to best.	Very good.	Good to very good.	Good.	Total, good to best.	Fair to good.	Fair.	Medium.	Poor.	Total, poor to best.	Percentage of good or better.
Very good to best X very good				2	9	11	4	7		1	23	48
Very good X very good		1	7	5	22	35	8	24	3	14	84	42
Very good X good to very good			2	8	30	40	9	30	2	20	101	40
Very good X good				3	6	9	3	43	7	23	85	10
Good to very good X good to very good			1	6	43	50	20	48	6	36	160	31
Good to very good X good			1		2	3	8	38	2	15	66	4
Good X good		1	5	3	31	40	18	59	15	31	163	24
Good X fair				1	3	4	1	6	1	4	16	25
Fair to good X poor					2	2	5	9	1	23	40	5
Medium X medium			1	3	18	22	14	54	20	103	213	10
Poor X poor				1	1	2		7	2	40	51	4
Total number of progeny		2	17	32	167	218	90	325	59	320	1,002	21

The tendency for the proportion of seedlings of good quality to decrease as we use parents of poorer quality shows clearly the importance of breeding from varieties of only the highest excellence, and even then we must be reconciled to a relatively small percentage of seedlings of good quality.

Practically every grape in the vineyards of the New York Station which ranks high in quality possesses some blood of *V. vinifera*. A moment's consideration of the history of the species shows us the reason for this predominance. European grapes are centuries removed from the wild and have been subjected to a more intense selection than any other fruit; the "level of mediocrity" has been raised to such a point that the species has become a powerful factor in transmitting high quality.

In this connection it is well to speak again of the future that lies ahead of the breeder who will search out and use those varieties of this potent species which blend best with our hardy native kinds. The ages of selection and breeding in Europe have developed varieties of this one species adapted to nearly as wide a range of climate as is covered by all our native species taken together. The proper selection of parents among these should enable us greatly to extend and enrich our viticulture.

A considerable proportion of the seedlings the results of whose crossing are given in Table V are pure seedlings. These have been separated and tabulated in Table VI. Comparing the percentage of those pure seedlings which are good or above good in quality with the percentage of the remaining similar cross-bred seedlings shows an interesting condition. The pure seedlings are uniformly poorer in quality than the crossed seedlings. Is this due to the decrease in vigor which seems to follow selfing, or is there some weightier reason?

TABLE VI.—*Quality of pure seedlings of grapes*

Parental type.	Types of progeny.				
	Good or above good.	Below good.	Total.	Percentage of good or above.	Percentage of crossed seedlings good or above
Very good × very good	14	25	39	36	47
Good to very good × good to very good	6	26	32	19	34
Good × good	33	102	135	24	25
Medium × medium	17	147	164	10	17
Poor × poor	0	31	31	0	19
Total	70	331	401	17	31

SIZE OF BERRY

In order to economize space it was necessary to plant the seedlings so close together in the test vineyards that the clusters frequently did not reach full and characteristic size. For this reason the size of cluster can not be discussed, although it is an important factor. The size of the berry, on the other hand, is one of the size factors least influenced by environment and season. The data from these vines should be of value and are presented in Table VII.

TABLE VII.—*Inheritance of the size of the grape berry*

Parental types.	Classes of seedlings.						
	Very small.	Small.	Below medium.	Medium.	Above medium.	Large.	Very large.
Large × large	1	2	3	[a] **28**	[a] **28**	19	8
Large [b] × medium to large [c]	1	6	7	56	34	[a] **67**	6
Large × medium	.....	1	.....	[a] **4**	2	2	.....
Medium to large × medium to large	5	34	35	[a] **103**	59	20	4
Medium to large × medium	.....	20	35	[a] **57**	37	3	2
Medium × medium	4	49	39	[a] **83**	38	11	.....
Medium to large × small	.....	[a] **13**	11	12	12	3	.....
Medium to small × medium to small	26	[a] **35**	12	23	7	.....	.....
Small × small	5	[a] **16**	4	5	3	.....	.....

[a] Numbers in the bold-face type represent the mode.
[b] The reciprocal is included in each cross.
[c] The use of the two terms shows that the berries varied from medium to large in the same variety.

A study of the various crosses which have entered into Table VII has failed to show any indication of purity for size among the varieties studied. Lacking exact measurements for the various sizes, it is not possible to compute an accurate mean, but the relative position of the mean with respect to the mode can be determined by a short study of the table. The wide variation about the mean, even in crosses where both parents were of the same size, prevents the only cross made between extremes of size, medium to large × small, from showing any clear tendency for the F_1 progeny to be intermediate. The steady decrease in the mean and mode as the parental types grow smaller shows clearly the strong tendency for a variety to produce progeny centering around its own size.

FORM OF BERRY

The ovalness of many varieties of *V. vinifera* is so pronounced that some have given this as a species characteristic and have assumed that ovalness in our American grapes was an indication of the presence of blood of this species, an assumption hardly warranted by the facts. The large number of markedly oval varieties among table varieties of *V.*

vinifera, together with the complete or nearly complete loss of this extreme form in hybrids with our American grapes, would lead us to suppose that this pronounced ovalness is perhaps a nearly pure form and that it is either recessive to roundness or else unites with roundness to produce a less pronounced oval. It is this latter type of oval that is referred to in Table VIII showing the inheritance of berry form. The appearance of so many seedlings with round berries in crosses of such oval varieties would tend to strengthen the idea that this is an intermediate form.

Any study of oblateness is made uncertain by the small number of varieties that possess this form. One of the most pronounced is the Goff, a seedling originated at this Station. The behavior of pure seedlings of the Goff grape would seem to indicate that, in this variety at least, oblateness is a pure form and its disappearance when combined with round, as is shown in Table VIII, would seem to show it as recessive to round

TABLE VIII.—*Inheritance of form of the grape berry*

Parental types.	Types of progeny.						
	Oblate.	Slightly oblate.	Oblate to round.	Round.	Round to oval.	Slightly oval.	Oval.
Oval × oval	.....	1	1	[a] 15	7	2	11
Oval × round to oval	.....	.....	.....	[a] 56	10	15	10
Round to oval × round to oval	3	3	10	[a] 129	30	25	56
Round × oval	.....	.....	.....	[a] 15	1	6	3
Round × round to oval	.....	1	2	[a] 100	9	14	1
Round × round	10	17	22	[a] 333	34	24	17
Round to oval × round to oblate	3	.....	1	[a] 17	2	2	1
Round × round to oblate	.....	4	2	[a] 42	5	2	1
Round to oblate × round to oblate	3	.....	1	[a] 24	2	1	8
Round × oblate	.....	.....	.....	7	.....	.....	.....
Oblate × oblate	[a] 15	.....	.....	1	.....	.....	.....

[a] Numbers in bold-face type represent the mode.

From a study of Table VIII it is seen that the mean would be more nearly coincident with the mode in each cross than was the case in Table VII. This shows clearly the strong tendency for roundness to obscure both oval and oblate.

SEASON OF RIPENING

The period of ripening of a variety depends so much upon the vigor of the vine, the season, cultural methods, and environmental conditions that no very accurate data can be presented. In one year all varieties may be 10 days earlier than normally, while in another year early varieties

may be unusually early; but a cold, wet period late in September and early in October may cause the late varieties to be unusually late. These variations are minimized when the records extend over a number of years. The ripening dates of the seedlings are usually taken for at least three years—not long enough, but much better than if taken for a single year.

In Table IX the ripening season extends approximately through the months of September and October. The first two periods cover about 15 days each, the next two about 10 days each, while the length of the last period is usually fixed by the first killing frosts.

TABLE IX.—*Effect of heredity on season of ripening of grapes*

Parental types with reference to ripening season.	Ripening periods of progeny.					
	Approximate mean.	Very early.	Early.	Early midseason.	Midseason.	Late.
Early X early	Sept. 23	8	[a] 30	20	.....	.....
Early X early to midseason [b]	Sept. 22	13	[a] 46	18	.....	.....
Early to midseason X early to midseason	Sept. 27	7	[a] 46	42	7	1
Early X midseason	Sept. 28	.....	20	[a] 22	.....	.....
Early to midseason X midseason	Sept. 26	21	[a] 126	100	8	.....
Midseason X midseason	Oct. 1	11	165	[a] 244	49	.....
Early X late	...do.....	.....	8	8	.....	.....
Early to midseason X late	Sept. 27	2	[a] 9	2	.....	3
Midseason X late	Oct. 4	3	20	[a] 27	18	6
Late X late	Oct. 7	.....	10	[a] 104	19	14

[a] Numbers in boldface type represent the mode.
[b] Each cross includes also the reciprocal.

As would be expected, Table IX fails to show purity or dominance for any one season, but it does show, both in the mode and in the approximate mean, the extent to which the season of the parent influences the offspring. A study of the varieties which enter into the table has failed to show results at all different from those of the group in which they fall.

NEW VARIETIES FROM EARLIER CROSSES

The results of the first 20 years of work were anything but encouraging. Now, however, there is tangible evidence that progress is being made. A vineyard of 1,500 seedlings bred from 1898 to 1903 has by a process of vigorous selection decreased to less than 75 vines, but among this number are several that seem very promising. Five of these have already proved so desirable both at Geneva and in a test vineyard at the Station's Vineyard Laboratory at Fredonia, N. Y., that in the fall of 1914 it was decided to give them names and place them in the hands of the nurserymen.

SUMMARY

In the last 25 years, during which time nearly 10,000 seedlings have been grown, various changes in the methods used at the Geneva Experiment Station have been made as the knowledge of breeding laws has been extended.

Results have compelled the belief that improved varieties of grapes will not be produced to any extent until the fundamental laws of heredity are understood. The present aim is to discover these laws.

The work is now progressing mainly along two lines: (1) The determination of the breeding possibilities of varieties of grapes and (2) the study and interpretation of breeding phenomena.

Nearly 200 varieties of grapes have been used in the breeding work.

Much of the value of the early work was lost by growing too few seedlings of each cross.

Recently *Vitis vinifera* has been used to a considerable extent in hybridization.

The usual method of emasculation has been ineffective in a few cases and may be open to criticism.

One of the surprises in the study of grape varieties was the failure of many commercial sorts to transmit desirable qualities.

In order to study grape varieties, nearly 3,000 selfed, or pure, seedlings have been grown. These are uniformly lacking in vigor.

The inheritance of those grape characters which have sufficient data available has been discussed in this paper.

Of the two types of stamens, reflexed and upright, the first is correlated with complete, or nearly complete, self-sterility, the second with self-fertility.

Self-sterility is probably caused by impotent pollen. It exists in varying degrees and depends to some extent upon the condition of the vine and environmental factors.

Self-sterile varieties of grapes being undesirable from a horticultural standpoint, can we eliminate those with reflexed stamens? The following crosses have given upright and reflexed stamens in the indicated ratios:

$$U \times U = 4.3\ U{:}1R$$
$$R \times R = 1.2\ U{:}1R$$
$$R \times U = 1\ U{:}1R$$
$$U \times R = ?$$

Breeding from the upright varieties only will decrease but not eliminate the seedlings with reflexed stamens.

The following seem to be the results secured in the study of sex inheritance:

Hermaphrodite female × hermaphrodite male = all hermaphrodites.

Hermaphrodite female × pure male = ½ hermaphrodites + ½ males.

Two general laws have been formulated with regard to color of the skin: (1) White is a pure color; and (2) it is recessive to both black and red.

No black variety has proved pure for blackness. Some contain white, others white and red. Red varieties are equally diverse.

The colors of pure seedlings of certain varieties show wide variation, even when derived from varieties of similar color.

In the inheritance of quality the most noticeable thing is the low percentage of seedlings whose quality is good or above good. This is probably due to the leveling influence of the wild ancestors from which the seedlings are but a step removed.

Most grapes of high quality possess some *V. vinifera* blood. This predominance of high quality is probably due to the intense selection to which the species has been subjected for centuries.

The pure seedlings on the New York Station grounds have been lower in quality than crossed seedlings.

In the inheritance of size of berry there is no indication of dominance of any one size, though there is a tendency for a variety to produce seedlings approaching its own size.

The oval form of many *V. vinifera* hybrids is probably an intermediate between round and a more pronounced oval. Oblate may be a pure form recessive to round.

The season of ripening of the parent influences to a considerable extent the season of the offspring.

A vineyard of 1,500 seedlings bred from 1898 to 1903 has dwindled through selection to less than 75. Of these, 5 have already proved promising enough to be named.

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ASCOCHYTA CLEMATIDINA, THE CAUSE OF STEM-ROT AND LEAF-SPOT OF CLEMATIS

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INTRODUCTION

The sudden dying of clematis plants has been known for many years, and there has been much speculation as to its cause and prevention. Apparently the disease occurs in both Europe and America wherever the large-flowered kinds of clematis are grown extensively. From published accounts it is clear that the various writers had in mind the same disease, though they ascribed it to different causes. In 1884 Arthur (1)[1] studied a clematis stem-rot which he suspected of being due to the fungus *Phoma clematidis* Sacc. Trelease (16), Comstock (3), Klebahn (7), and others have considered nematodes as the causal agent. In specimens received from Klebahn, Ritzema Bos (2) found nonparasitic nematodes, while in material of his own collection he found the larvæ of a fly, *Phytomyza affinis*, and a species of Pleospora. Prillieux and Delacroix (9) and Morel (8) believed the disease to be of bacterial origin. Sorauer (13) reports a gall-like formation on the stem of *Clematis jackmanni* near the surface of the soil and attributes the death of the affected plants to *Gloeosporium clematidis*. Green (5, p. 284–285) has reported the relative susceptibility of a few varieties of clematis which he grew, but he did not attempt to ascertain the cause of the disease. Except for a preliminary abstract by the writer (4), the primary cause of the clematis disease has heretofore been unknown.

DESCRIPTION OF THE DISEASE

The clematis disease manifests itself differently on the various species and hybrids. On hybrids grown in the field it is a stem-rot, while in the greenhouse, where the cuttings are propagated, it is a leaf-spot as well as a stem-rot. On *Clematis paniculata* the disease takes both forms.

C. paniculata, a type species, is propagated from seed[2] and when grown in uninfected cold frames or greenhouses remains free from disease. Such seedlings are either potted or placed in beds, where they are planted about an inch apart in rows 4 inches apart. In the fall, when these plants

[1] Reference is made by number to "Literature cited," p. 341–342.

[2] For description of general methods of propagation and of the various species of clematis see the following: Bailey, L. H., ed. Cyclopedia of American Horticulture. ed. 7, v. 1, p. 327–333, fig. 485–492. 1910. Le Bêle, Jules. The clematises. *In* Garden, v. 53, no. 1388, p. 544–548, illus.; v. 54, no. 1391, p. 39–40, no. 1395, p. 127; no. 1396, p. 138; no. 1399, p. 200–201; no. 1401, p. 240–241. 1898. *Translated from* Bul. Soc. Hort. Sarthe, 1896.

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have made a growth of 8 to 10 inches, the leaf-spot may make its appearance and be thus carried over the winter on the dead leaves or in lesions formed on the vines. If these plants are left in the beds a second season, the fungus may make its appearance early in spring and increase until by midsummer no vine is wholly free from disease. The leaf-spot may first appear either as a mere dot or as a water-soaked area. With the advent of moist warm weather the former usually leads to the latter. On drying the water-soaked spot becomes tan-colored with a red margin. Plate L shows the general appearance of the disease on *C. paniculata*. The older leaves are badly diseased or dead, and the fungus has grown down the petiole to the node, where in time the vine may become girdled. The younger leaves show the early stages of the leaf-spot. The stem shows the lesions, reddish in color, formed at the nodes and on the internodes. Later these take on a gray color. Plate LI illustrates a group of leaves of *C. paniculata* with spots that are zonate, owing to the unequal growth of the fungus under the influence of changes in temperature. The newly formed spot has a dark margin of red tissue and a lighter center. Pycnidia are produced on the diseased leaves. Succulent growing tissues succumb more readily to the disease than do the woody stems. In the latter it may require a month for the fungus to pass a node. Plate LII, figure 1, shows a portion of a vine of *C. paniculata* 44 inches long on which the lower leaves were wilted, while the distal ones were still turgid. The fungus entered through the stub *a*. It girdled the stem and disintegrated the upper roots, leaving the central cylinder as the only means of communication with the healthy roots below. Pure cultures of *Ascochyta clematidina* were obtained from the boundaries of the lesion. Pycnidia were formed on the stem above the ground. In other cases pycnidia have been found on the epidermis, while the tissues underneath were healthy.

Some of the large-flowered kinds of clematis are grown from seed, but in America the majority of those cultivated are hybrids. They are propagated from cuttings taken from rapid-growing, disease-free vines. The cuttings are made in May or June and consist of a single node with the attached leaves and the internode below. They are placed in moist sand and exposed to bottom heat or else grown in forcing frames. In forcing frames the humidity and temperature are usually higher than is found in the average greenhouse. Under these conditions, if the spores are present, a leaf-spot may be formed, and the entire cutting may be killed or the fungus may be halted at the node. The fungus that has been checked may again become active when the cuttings are potted and placed in the greenhouse, or new infections may take place on the leaves. In the fall some of the plants are placed in storage, while others are kept over winter in the greenhouse and the tops used for cuttings. In the following spring both lots are transplanted into the open field and, unlike *C. paniculata*, are not allowed to trail on the ground. Experience has

taught the nurseryman that supported vines, owing to the better ventilation they receive, do not die as readily as those left on the ground. They make a vigorous growth, and yet when about to bloom they may suddenly die. It is at this stage that the disease first attracts the attention of the nurseryman, though in reality it was on the plants while they were still in the greenhouse and was there overlooked. Plate LIII, figure 1, shows a plant free from leaf-spot, yet girdled by the fungus lurking in the stub *a*, which in ordinary practice is not removed. Plate LIII, figure 2, is a reproduction of another vine of *C. jackmanni* that had many pycnidia of *A. clematidina* on the old stub. After the removal of this stub some of the discolored tissue still remained. The new shoot formed is wilting, and the split stem shows discolored fibrovascular bundles from which *A. clematidina* was isolated. In advanced stages the roots may disintegrate similarly to that shown in Plate LII, figure 1. The spots on the leaves of *C. jackmanni* resemble those found on *C. paniculata*, *C. recta*, and *C. virginiana*.

ISOLATION OF THE CAUSAL FUNGUS

By previous writers the dying of clematis plants has been assigned to various factors, but none have discovered the primary cause. The dying of the leaves owing to lack of light, the breaking of the vine by strong winds, and injury by nematodes are factors that have been eliminated as primary agencies, while the constant association of *A. clematidina* points to it as the causal organism. The fungus can be readily isolated by the poured-plate method, using the spores from a crushed pycnidium, by the use of sterile leaf tissues, or by the use of free-hand sections of diseased material. The last-named method consists in making free-hand sections under as sterile conditions as possible by sterilizing the outer tissue and the instruments. If such sections show mycelium they are transferred to sterile media. Some have maintained that no mycelium can be seen in the decayed tissue, but the writer has observed in the tissues 3 to 5 mm. from the boundaries of the lesions mycelium which in plate cultures proved to be that of the causal organism, *A. clematidina*.

A. clematidina grows well on the media generally used in the laboratory. It grows at about the same rate on nutrient glucose agar, oatmeal agar, bean pods or stems, moist oats, and corn meal, producing pycnidia in five to seven days. These pycnidia may show a pink tinge at first and later turn brown. The fungus grows less vigorously on corn-meal agar, potato agar, starch agar, sugar-beet plugs, apple twigs, and sterile raw carrot. Oatmeal and starch agar are at first turned green, but later take on a brown color. On starch agar the mycelium penetrates the medium and forms chlamydospores, as shown in Plate LII, figure 1. These are thick-walled, green-brown bodies filled with oil globules. When placed in water, they germinate readily.

INOCULATION EXPERIMENTS

To prove the pathogenicity of *A. clematidina*, mycelium from pure cultures was inoculated into stems of *C. paniculata* and *C. jackmanni*. In all cases lesions were produced, while the checks remained normal. From such lesions the fungus was reisolated, and, when again inoculated into either host, typical lesions were produced. In all, four sets of inoculation experiments were carried out at various times, making from 3 to 10 inoculations on each of 32 plants. Inoculations on succulent stems caused the vines to wilt in four days, while in one case an inoculation on a woody vine 6 mm. in diameter required 47 days to kill the plant. Pycnidia were produced on all lesions.

Plants of *C. paniculata* were sprayed with sterile water containing spores of *A. clematidina* and then kept under bell jars for two days. On the third day the leaves showed water-soaked spots of various sizes, while the checks, which had been sprayed with sterile water, remained free from disease. To test the effect of temperature on infection, two plants were sprayed with the same spore-laden water and then subjected to different temperatures: 23° C. and 10° C. At the end of five days the plant kept at 23° showed 45 leaf spots, while the plant kept at 10° showed but 1 spot.

Spores placed on the lower surface of the leaves produced more spots than those placed on the upper surface. Typical lesions were also produced on the roots by inoculating them with the mycelium from a pure culture.

The *A. clematidina* isolated from *C. paniculata* was inoculated into growing stems of bean, pea, muskmelon, pumpkin; into stems, petioles, and fruits of eggplant (var. Black Beauty); and into the young shoots of elm. In all cases negative results were obtained. On most of these plants pycnidia were produced on the tissues killed in making the wound, but in no case did the mycelium penetrate the healthy tissues and form a lesion.

TAXONOMY OF THE FUNGUS

Arthur (1) observed a species of Phoma, possibly *P. clematidis*, on clematis, but on consulting the original notes made by him it is clear that he had a fungus different from that found by the writer. On but one occasion has *Phoma* sp. been found and that was a saprophyte on the leaf of *C. paniculata*. It was isolated in pure culture, the mycelium inoculated into the stems, and the spores sprayed on leaves, but in no case were lesions or leaf-spots produced.

Saccardo (11) notes *A. clematidina*, *A. vitalbae*, *A. indusiata*, and *A. davidiana* as occurring on various species of clematis, and their chief point of difference is in the size of the spores. The writer has examined the specimens of *A. clematidina* Thümen on *C. virginiana* collected by Mr. J. J. Davis in Wisconsin and distributed in Fungi Columbiani No.

2503; also those of *A. indusiata* Bres. on *C. recta* in Krieger's Fungi Saxonici No. 1189. In both, the spots resemble those found on *C. paniculata* and *C. jackmanni*. In the former the spores are cylindrical 1-septate and hyaline. They measure 8 to 12 by 3.2μ, the average dimensions being 9.5 by 3.2μ. The spores of the latter species are hyaline to honey-colored, somewhat constricted, and measure 12 to 22 by 6.3μ, with an average of 19 by 6μ.

The writer has repeatedly examined the species of Ascochyta on clematis and found it quite variable. The chief difference is in the spores, though sometimes the pycnidia are more deeply immersed than at other times. Plate LIV, figure 1, shows a pycnidium in the leaf tissues of *C. paniculata*. The spores vary in length from 6 to 28μ and in width from 3 to 6μ, but generally they are about 9 to 13 by 3 to 4μ. Plate LII, figure 3, shows the typical spores. The spores are either 1- or 2-celled, rarely 3-celled. Some leaves of *C. jackmanni* collected in the fall of 1914 showed pycnidia having spores as long as 28μ and averaging 18 by 5.7μ. Inoculations with material from cultures obtained by the isolation of single spores showed that this fungus was the same as that usually encountered. The various differences in color shown by the spores disappear when the spores are plated out under control conditions. Considering the variability of the fungus found by the writer, any of the descriptions given for the different species of Ascochyta described on clematis would in general apply to it. Hence, the name selected is the oldest one, *Ascochyta clematidina* Thümen, the description of which is here emended as follows:

Ascochyta clematidina (Thümen).

Ascochyta clematidina Thümen, Pilzfl. Sibir. n. 619, 1884, *in* Sacc. Syll. Fung., v. 3, p. 396.

On stems and foliage; spots circular, zonate to indefinite; pycnidia (on leaves mostly epigenous, sometimes hypogenous) tan to dark brown, scattered to gregarious, globose to subovoid, immersed, then erumpent, ostiolate, averaging 120μ in diameter; spores variable, subellipsoidal to cylindrical, 1- or 2-celled, septa more or less medial, sometimes constricted, hyaline to dilute honey or olive color, often guttulate, 6 to 28 by 3 to 6.4μ, usually 9 to 13 by 3 to 4μ; exuded spore mass honey-colored, sometimes pink.

On living leaves and stems of *Clematis paniculata*, *C. virginiana*, and the hybrids *C. hendersoni*, *C. henryi*, *C. jackmanni*, *C. ramona*, *C. Duchess of Edinburg*, *C. Mme. Baron Veillard*, and *C. Mad. Èdouard André*. According to Von Thümen, it occurs also on living leaves of *C. glauca*. As yet no perfect form of *A. clematidina* has positively been found.

CONTROL EXPERIMENTS IN 1913

In 1913 some 2-year-old plants of *C. paniculata* that had made a dense, matted growth of tangled vines were badly diseased, while a bed of seedlings next to them was free from disease. In an attempt to save the 2-year-old plants, they were cut back to a length of 4 to 6 inches and then sprayed with Bordeaux mixture on July 21. A small area was left unpruned and unsprayed as a check. By October 17 the seedlings,

which had made a growth of 8 to 10 inches, showed an occasional leaf spot. The pruned-and-sprayed plot produced an excellent growth, but had some leaf-spot and a few girdled vines. The check showed many dead plants, and none of the living ones were entirely free from disease.

CONTROL EXPERIMENTS IN 1914

A writer in Garden (10) states that Bordeaux mixture, when applied to diseased clematis plants, was of no benefit in checking the disease. In 1914, spraying experiments were carried out by the writer on 18 rows (about 300 feet long) of plants of *C. jackmanni*, half of which were sprayed with Bordeaux mixture (4-4-50 formula), while the others were left as checks. Four of these, two checks and two sprayed rows, were pruned on June 12 and 25 in such a manner as to remove the dead stubs of the previous year. Plants from which all of the discolored tissue could not be removed without injury to the entire vine were marked with tags. The rows receiving Bordeaux mixture were sprayed every two weeks. The final examination was made on October 19. No difference could be seen between the sprayed and the check rows either in the amount of leaf spot or the number of dead plants. The same held true for the pruned and unpruned rows. However, there was but little leaf-spot, and it was observed that the dead plants in the pruned rows were invariably plants that had been tagged. No doubt the pruning was done too late in the season to be of any benefit. Sulphur dusted on cuttings in the forcing frames did not check the disease. Plants in the greenhouse sprayed with soap-and-sulphur mixture so as to cover the leaves with a thin film were healthier than the unsprayed plants. These, however, were not carried through the second season, and hence the ultimate results are unknown.

Two long, narrow beds of *C. paniculata* were utilized for spraying and dusting experiments in 1914. Bed 1 consisted of yearling plants untreated in 1913. Bed 2 contained 2-year-old plants pruned and sprayed with Bordeaux mixture in 1913. Both beds were divided into plots 6 by 25 feet in size.

Two plots in each bed were sprayed with Bordeaux mixture six times at intervals of two weeks from May 15 to August 8. On the same dates one plot in each bed was sprayed with a soap-and-sulphur mixture composed of 1 pound of soap, 6 pounds of sulphur, and 15 gallons of water. Two and one-half gallons of the mixture, containing 1 pound of sulphur, were used on each plot at each application. On two plots in bed 1 and one plot in bed 2 the plants were dusted six times with sulphur, using 1 pound to the plot at each application. The remaining eight plots (three in bed 1 and five in bed 2) were left untreated for checks.

As the season advanced, the virulence of the disease increased, becoming quite severe on all three check plots in bed 1 and one check plot in bed

2. On August 8 some of the plots were pruned, thus terminating the main experiment. The effect of the different kinds of treatment up to August 8 is shown in Table I.

TABLE I.—*Results of an experiment on the control of leaf-spot and stem-rot of Clematis paniculata*

Bed No. 1.			Bed No. 2.		
Plot No.	Treatment.	Percentage of plants free from disease.	Plot No.	Treatment.	Percentage of plants free from disease.
1	Bordeaux mixture	75	9	Bordeaux mixture	60
2	Check	2	10	Check	2
3	Bordeaux mixture	80	11	Sulphur	70
4	Check	10	12	Check	45
5	Sulphur	80	13	Soap and sulphur	100
6	Soap and sulphur	100	14	Check	85
7	Check	10	15	do	85
8	Sulphur	65	16	do	85
			17	Bordeaux mixture	95

The results are more uniform in bed 1 than in bed 2. This may be due to the treatment of bed 2 the previous year. That there was more disease in plots 9 and 10 than in the other plots of bed 2 may be accounted for by the fact that these two plots were used as checks in 1913, and hence were neither pruned nor sprayed in that year. Plots sprayed with the soap-and-sulphur mixture remained free from leaf-spot and lesions on the stems; hence, their condition is rated at 100 per cent. In rating the other plots the amount of leaf-spot, the number of lesions, and number of girdled plants have all been considered.

On the plots 1, 3, and 17, which were sprayed with Bordeaux mixture and which were not pruned on August 8, the spraying was continued to the end of the season. Check plots 4, 14, and 16 also were left unpruned. On October 19, when the final examination of these plots was made, it was found that in plots 1 and 3 the leaves on the new growth were disease-free and that there was but an occasional dead vine. On check plot 4 half of the newly formed leaves were diseased, and about one-third of the vines were dead. Plots 16 and 17 showed about the same amount of leaf-spot, only an occasional spot. The former, however, showed more lesions on the stems than the latter. Check plots 2, 7, 10, and 12, which had been pruned back to a length of 4 to 6 inches and then given one application of Bordeaux mixture, had but little disease as compared with plot 4, which had received no treatment whatsoever.

INJURIOUS EFFECTS OF SULPHUR ON CLEMATIS PANICULATA

The promising results obtained by Smith (12) in dusting asparagus with sulphur for the control of rust led the writer to try sulphur in con-

trolling the fungus on clematis. Up to August 8 the results were satisfactory, and no injury was observed. Soon after the pruning of August 8 there were several hot, dry days followed by a period of rainy weather, during which water accumulated at the end of bed 1. Up to the end of the season only one plant in plot 8 had sent forth a new shoot. The other vines were dead, and the stems at the surface of the ground for about an inch were discolored. A particle of soil placed on the tongue had an acid taste. According to a test made by Mr. R. F. Keeler, 1 gm. of this soil is equivalent to 0.5 c. c. of 0.1 *N* acid, while soil from the adjoining check plot 7 was neutral. In check plot 7 a few vines died, owing to the lack of drainage, but it seems apparent that in the other cases the injury was caused by sulphur that had washed from the foliage and had accumulated in the upper layer of soil. As the season advanced, sulphur injury was observed in the other treated plots, but in these cases the injury was localized in areas not larger than 2 feet in diameter. The injury began to show on the plots sprayed with the soap-and-sulphur mixture after nine applications had been made, while in the plots dusted with sulphur it appeared after six applications had been made.

SOAP AND SULPHUR AS A SPRAY MIXTURE

A mixture of about 1 pound of laundry soap and 6 pounds of sulphur in 15 gallons of water was in common use as a greenhouse spray at the nursery where the spraying experiments were conducted. It was used with success in the control of leaf-blotch, *Diplocarpon rosae* Wolf, on susceptible varieties of roses grown in the forcing houses. Halsted and Kelsey (6) used Ivory soap at the rate of 1 ounce to 4 gallons of water for spraying *Phlox drummondii* and the common verbena attacked by powdery mildew and were able to check it. Another (15) has shown that soap at the rate of 1 ounce to 1 gallon of water controlled the mildew and aphids of roses. R. E. Smith (12) recommended that, in the absence of dew, whale-oil soap be sprayed on asparagus tops to hold the sulphur that is to be dusted over them for the control of the rust. Spieckermann (14) has shown that weak solutions of soap have a nutritive value and can be assimilated by the higher fungi.

In order to test the toxic effect of soap, mycelium of *A. clematidina* was transferred to Petri dishes containing soap agar of different strengths—viz, 2 per cent agar containing alkali-free Ivory soap in the proportion of 1 pound to 5, 10, 15, 20, and 40 gallons of the medium. Fifteen c. c. of such media were placed in each Petri dish. When the fungus became established, the diameter of the culture was measured daily, and the rate of growth was considered as a measurement for toxicity. Cultures on 2 per cent agar and nutrient-glucose agar served as checks. Table II gives the averages of growth of four or five cultures on each medium grown under the same conditions at room temperature.

TABLE II.—*Summary of the data on the toxic effect of soap agar of various strengths on Ascochyta clematidina*

Culture medium.	Number of cultures.	Average diameter of cultures after growth for—									
		3 days.	5 days.	7 days.	9 days.	11 days.	13 days.	15 days.	17 days.	20 days.	23 days.
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
Soap agar (1 lb. to 5 gals.)	5	0	0	0	0	0	0	0	0	0	0
Soap agar (1 lb. to 10 gals.)	5	0	0	0	0	0	0	0	0	0	0
Soap agar (1 lb. to 15 gals.)	4	0	7	9	11	15	18	20	22	25	28
Soap agar (1 lb. to 20 gals.)	5	0	11	15	19	23	26	30	34	39	44
Soap agar (1 lb. to 30 gals.)	4	8	20	25	32	40	44	50	56	67	73
Check, 2 per cent agar	5	10	21	29	40	51	61	70	77	85	.....
Check, nutrient-glucose agar	5	15	29	38	48	60	69	77	.....	.....	.....

Plate LIV, figure 2, shows a culture of *A. clematidina* on soap agar. The concentric ring or oily film about the culture may be 2 to 7 mm. wide. Upon the drying of the medium, crystals of stearic acid are seen only in this region, indicating that an active principle given off by the fungus liberated the fatty acid. The culture shows a green discoloration, which is due to the formation of brown-green, thick-walled chlamydospores. These bodies, as well as the mycelium, are filled with oil globules, while none are found in the 2 per cent agar. These cultural experiments indicate that soap at the strengths in which it is used as a contact insecticide has in itself fungicidal value, as well as being a means of adhesion or suspension for other materials.

METHODS OF CONTROLLING THE FUNGUS

The suggestions here given for controlling *A. clematidina* are based upon the observations and experiments made in the last three years. Greater success can be attained by changing the methods of culture than by spraying. Long experience has taught the nurseryman that there is less disease when the hybrids are supported while growing in the field or in the greenhouse than when they are permitted to trail on the ground. This holds true also for *C. paniculata*, but its selling price does not warrant so much expense for labor. This can be overcome by transplanting the plants from the beds to the open field after the first year, placing them far enough apart to prevent matting. Spraying is beneficial to such plants, but before making such applications it is advisable to remove all diseased leaves and dead vines. Plants so treated are disease-free in the fall. If seedlings are grown in a greenhouse where clematis has never been grown before and are kept away from older diseased beds, they will

remain disease-free. The fungus can live as a saprophyte on dead vines kept out of doors in baskets, and under such conditions it has lived over two winters, producing pycnidia and viable spores in abundance. This indicates that the same beds should not be used for clematis in successive years.

On hybrids the disease is primarily a greenhouse trouble and can be overcome by the use of cuttings made from healthy plants. A light spraying with the soap-and-sulphur mixture has proved satisfactory in the greenhouse. It could readily be applied also in the forcing frames. Diseased leaves or stubs should be removed as soon as discovered so as to prevent the fungus becoming established in the tissues.

The retail purchaser of clematis can prevent the dying of plants by taking proper simple precautions. The plants should be placed in good soil, well drained and on a sunny exposure. As soon as the new shoots have formed the old vine tissue should be carefully cut away close to the new shoots, removing all traces of the brown, discolored wood in which the fungus is to be found. Proper ventilation is obtained by training the plants to a strong trellis.

SUMMARY

(1) The stem-rot and leaf-spot of clematis is caused by the fungus *Ascochyta clematidina* (Thümen.).

(2) The plants are killed by the growth of the fungus down the petiole into the stems, thus girdling the plant at the node. The stem may be girdled also by the lesions anywhere on the internodes. Dead stubs left on the vines are a means of holding the disease over a period of time. New shoots may be formed below the girdled region, but the downward progress of the fungus ultimately kills the plant if the diseased tissue is not removed.

(3) Overwintering out of doors does not kill the fungus in cultures or on dead vines. Whenever the temperature permits, the fungus resumes its growth.

(4) The fungus is readily isolated and grows well on the media generally employed in the laboratory.

(5) The disease has been successfully produced by inoculating *C. paniculata* and *C. jackmanni* with the mycelium from pure cultures. The fungus has been reisolated from such inoculations, and with it lesions were again produced on other vines similarly treated.

(6) *A. clematidina* is not related to other common species of the genus Ascochyta, for inoculations made in growing stems of bean, pea, muskmelon, pumpkin, eggplant, and the young shoots of elm gave negative results.

(7) Spraying the plants with spores will produce the leaf-spot. More spots are produced when the spores are placed on the lower surface of

the leaf than on the upper. A temperature of 23° C. is more favorable for the production of the leaf-spot than a temperature of 10° C.

(8) The matting of the vines produces a condition most favorable for the spread of the disease. Ventilation can be obtained by supporting the vines or by planting them far enough apart to prevent matting.

(9) On the hybrids the disease can be controlled in the forcing frames or in the greenhouse by the use of sprays. In the field, the spraying of hybrids properly supported is of little benefit.

(10) On *C. paniculata* spraying with a fungicide checks the disease. In the field the removal of diseased leaves and vines before spraying is of practical value in controlling the disease.

(11) Sulphur dusted on *C. paniculata* in large quantities may cause injury.

(12) A mixture of 1 pound of laundry soap and 6 pounds of sulphur to 15 gallons of water, when sprayed on cuttings in the greenhouse or on *C. paniculata* growing in the beds, controlled the disease.

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PLATE L

Clematis paniculata: Portion of vine showing the general nature of the leaf-spot.

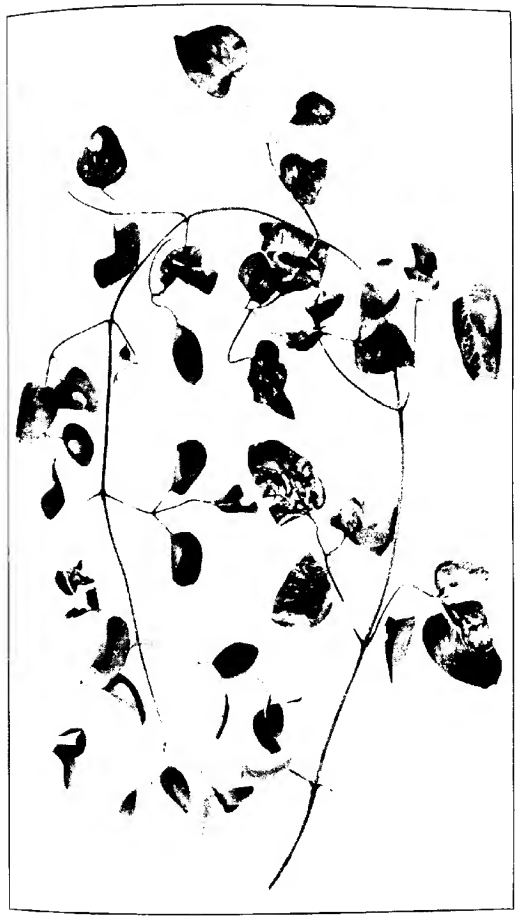


PLATE LI

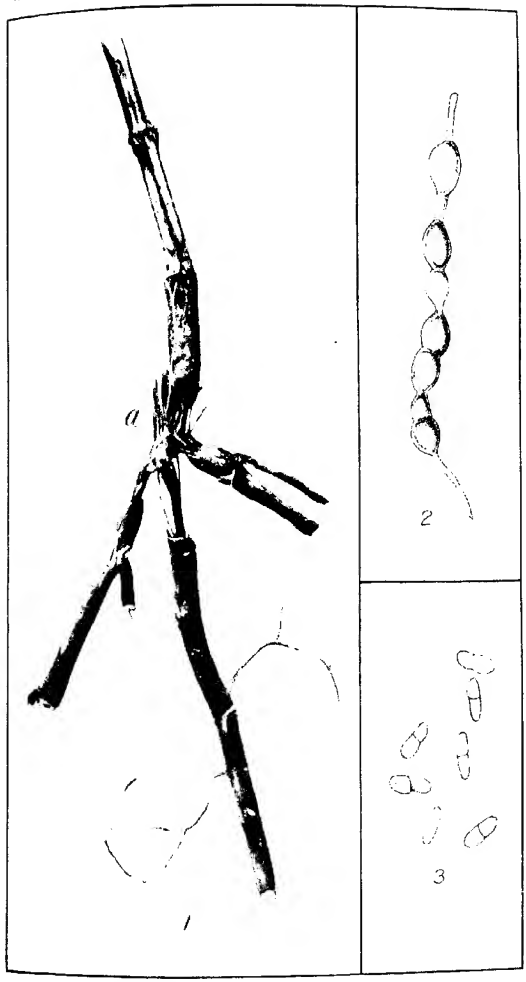
Clematis paniculata: Group of leaves enlarged to show the zonation and pycnidia of the spots. One leaf shows the newly formed spot, with its lighter center.

PLATE LII

Fig. 1.—*Clematis paniculata:* A portion of a vine 44 inches long that showed indications of wilting of the lower leaves while the distal leaves were still turgid. The fungus entered through the stub *a*. In the girdled region the parenchyma of the roots had disintegrated, leaving the central cylinder as the only means of communication with the healthy roots below. *Ascochyta clematidina* was isolated in pure cultures from the boundaries of the lesion.

Fig. 2.—*Ascochyta clematidina:* Chlamydospores as formed on starch agar.

Fig. 3.—*Ascochyta clematidina:* Camera-lucida drawing of spores.


a
1
2
3


a
a


a
b

PLATE LIII

Fig. 1.—*Clematis jackmanni:* A vine free from leaf-spot that has been girdled by *Ascochyta clematidina* in the region of the previous year's stub *a*. A new shoot would have been sent forth from an active bud at *b*, but it would have soon died, for the fungus had discolored the vascular bundles beyond this point. The presence of the fungus was proved by isolating it from the discolored tissue.

Fig. 2.—*Clematis jackmanni:* Plant from which the diseased stub *a* was cut away without removing the discolored tissue. The leaves were free from leaf-spot and were drying. The split stem shows the discolored fibrovascular bundles from which the fungus was isolated.

PLATE LIV

Fig. 1.—*Ascochyta clematidina:* Photomicrograph of a pycnidium from stained section of a leaf of *Clematis paniculata.*

Fig. 2.—*Ascochyta clematidina:* Culture growing on agar to which Ivory soap at the rate of 1 pound to 15 gallons of water was added, showing the oily film about the margin of the culture in which the crystals of stearic acid are found.

2

METHODS OF BACTERIAL ANALYSES OF AIR[1]

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INTRODUCTION

This study of methods of making bacterial analyses of the air was undertaken at the New York Agricultural Experiment Station in connection with the problem of determining the relation of the bacterial content of the stable air to the amount of bacterial contamination of milk before it leaves the cow stable. The latter problem forms part of a larger one which has been the subject of investigation at this Station for a number of years and which is now being completed in cooperation with the Illinois Agricultural Experiment Station. Briefly stated, this larger problem is a study of the relative importance of various barn operations in the production of sanitary milk. The work already reported can be found in bulletins of the New York Agricultural Experiment Station (8–11).[2]

In the present investigation two general methods of bacterial air analyses have been tested: One, methods of determining the number of bacteria in a given volume of air; and the other, methods of determining the amount of bacterial precipitation on a known area in a definite period of time. The greater part of the work, however, is devoted to a study of the former technique and involves the determination of the filtering efficiency of two aeroscopes frequently used by investigators and a modification of one of them.

HISTORICAL REVIEW

A brief review of the efforts of bacteriologists to devise satisfactory methods for the bacteriological analysis of the air will show the important stages in the development of the present technique. The methods which have been devised for quantitative purposes generally involve one of the following principles:

(a) Bubbling the air through a liquid, which acts as a filter. The liquid may be either nutritive or nonnutritive. In either case the liquid

[1] Credit is due to Dr. H. A. Harding, formerly bacteriologist at the New York Agricultural Experiment Station, for having suggested the original plan of the work and for valuable aid in carrying out the first part of it. Dr. Robert S. Breed bore a similar relation to the later part of the work, and assisted materially by his criticism of the manuscript. Dr. John Weinzirl, member of the Committee on Standard Methods for the Examination of Air, has also given several helpful suggestions during the course of the work. Mr. W. L. Kulp, Student Assistant in Bacteriology, made the series of analyses recorded in Tables III and IX. The writer wishes to express his gratitude for the aid thus given.

[2] Reference is made by number to "Literature cited," p. 366–368.

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is examined for bacteria by the usual methods employed for the bacterial analysis of liquids, which include direct microscopical examination, the plating of aliquot portions in a solid nutritive medium, and methods of fractional cultivation in liquid media. When the liquid filter is nutritive, it may be of such a nature as to harden on cooling. In this case the bacteria are allowed to develop in this medium.

(b) Drawing the air through porous solids, which act as filters. These filters may be either soluble or insoluble. According to its nature, the filter is either dissolved or washed in a sterile liquid and the latter analyzed for bacteria as indicated under 1.

(c) The bacteria in the air may be allowed to settle upon the surface of a solid nutrient medium and to develop into colonies wherever they fall. In using this method, a measured quantity of air may be used or the results may be expressed as the number of bacteria falling on a given area in a given period of time. The method may also be varied by using a sterile liquid instead of a solid culture medium, the liquid being afterwards analyzed to determine the number of bacteria present.

It is unnecessary to review the literature of air bacteriology before the time of Petri (20, 21) and Miquel (17, 18) in detail, since these authors have given us very full and complete accounts of the early work. As is well known, this early work had to do very largely with the efforts of Pasteur to convince the adherents of the theory of spontaneous generation that their supposed cases of life without previous life were really due to forms of life already existing in the air. Pasteur used primitive forms of our present methods of bacterial air analysis in several instances. Among these, he tried both soluble and insoluble granular solids as filtering agents, such as guncotton and asbestos. In his later work he used the vacuum-flask method of analysis for rough quantitative work. This method has been further developed by Hansen (7).

Miquel (17, 18, 19) also used several methods of analysis. Among these he tried an "aeroscope" containing a glass plate coated with a sticky mixture of glycerin and glucose which caught the bacteria, mold spores, dust, and the like. Since this technique did not allow a distinction between living and dead organisms, he devised a method of filtering air through water or other liquids and testing the liquids used for bacteria by means of the method of fractional cultivation. When taking samples at a distance from the laboratory, he used solid porous substances, such as glass wool, asbestos, and powdered sodium sulphate, as filtering agents.

Frankland (6) used finely powdered sugar, glass wool, and a mixture of glass wool and glass powder as filtering agents. Hueppe (14) and Straus and Wurtz (28) filtered air through liquefied nutrient gelatin and poured plates from this suspension. Von Schlen (25) used melted agar in a similar way.

In Petri's (21) first sand filter much coarser sand than that now employed was used and in deeper layers. Two 2.5 cm. layers of a sand which had been heated to redness and which would pass through a 0.5 mm. sieve were supported on the inside of a glass tube on wire-gauze disks. After aspirating a large volume of air through the filter tube by means of an air pump, the sand of each layer (the second acting as a control) was divided between a number of plates and mixed with nutrient gelatin. The number of colonies which developed after incubation was then counted and the number of bacteria originally present calculated from this.

Soper (26, 27) seems to have been the first to use two sand filter tubes in tandem. He washed the sand in sterile water and made his plates from this wash water instead of adding the sand directly to the Petri plates, as former investigators had done. In this way he obtained more transparent plates and probably a more uniform and thorough admixture than had his predecessors.

Winslow (33) compared the results from sand filters made of as coarse sand as that used by Petri with those from filters made of sand of 0.1 to 0.3 mm. in size, with much better results for the latter even when the layer of sand was reduced to 2.5 cm. as compared with 5.0 cm. of the coarser sand.

Weinzirl and Fos (30) were among the first investigators to use a very fine sand of standard size. Among the filtering agents which they employed were sands that passed through sieves with 100 and 150 meshes to the inch and mixtures of the former with powdered silica. They also tried varying the depth of the filtering layer from 0.5 to 2.5 cm. They found that, while the mixture of sand and silica was slightly better than the sand alone, sand which had passed through a 100-mesh sieve was very efficient. They also showed that a 1-cm. layer of sand was as efficient as a deeper layer. The number of tests recorded was small, and in the minds of some there was still a question as to whether or not lanes or pores that would allow the bacteria to pass through might not form in the sand. This point is discussed further on page 349 in connection with the present studies. The above results of Weinzirl and Fos formed the basis for the recommendation of the sand-filtration method by the Committee on Standard Methods for the Examination of Air (2).

One of the recent methods of bacterial air analysis which gave sufficient promise of usefulness to be considered by this committee is that of Rettger (22). This method, which is more fully described on page 348, is a modification of Miquel's method of filtering through distilled water (19) and consists of a device for finely dividing the air as it enters the liquid.

In 1912 a subcommittee (3) gave a report of progress in which they recommended certain modifications in the standard method given in their earlier report. The technique recommended in this later report

was the one which was tested in the present work. Weinzirl and Thomas (31) as members of this subcommittee made a series of comparative tests, using both the new standard method and Rettger's method, securing results slightly favoring the new standard method.

A number of men, other than those mentioned in this review, have contributed to the development of satisfactory methods of bacterial air analysis, among whom are Sedgwick and Tucker (24), William (32), Ficker (5), and others.

The method which has been most used in determining bacterial precipitation is the plate exposure method of Koch (15). He allowed a layer of gelatin to solidify on the surface of a plate in the bottom of a cylinder. The analysis consisted in exposing this layer of gelatin to the air for a known period of time and then allowing the germs to develop on the surface of the medium. Most investigators using the plate exposure method have dispensed with the cylinder, which was designed to prevent side currents of air from affecting the results, and have used Petri plates alone. Koning (16) used a similar principle in some of his analyses. He determined the precipitation per unit area by exposing a known volume of a sterile liquid to the air and analyzing the liquid afterwards.

Koch's method (15) has also been modified so as to relate it to a definite volume of air. Hesse (13) drew air slowly through a long glass tube lined on its inner surface with a layer of gelatin, upon which the bacteria and molds were deposited and allowed to develop.

Winslow (33) modified Hesse's method by substituting two bottles with a layer of gelatin on the bottom of each for the long roll tube. After drawing a liter of air into the system, the bacteria were allowed to settle on the gelatin surface. Weinzirl and Fos (30) agree with Winslow in regarding the method as unsuited for field work.

PRESENT STUDIES OF METHODS

TECHNIQUE

The material to be analyzed was plated in duplicate—or in triplicate in some cases—within one hour after sampling. The medium used was an agar made according to the formula now recommended by the Committee on Standard Methods for Bacterial Milk Analysis (1), except that the acidity was usually between 1.2 and 1.5 per cent normal acid to phenolphthalein. The plates were incubated in a constant-temperature incubator for five days at 18° C. (later 21° C.) and then for two days at 37° C. Except in a few instances, check plates were made which were designed to test out the sterility of the filter tubes, water blanks, and Petri plates. In a few cases where the check plates contained more than the occasional colonies which appear as a result of accidental contaminations, the entire results of the tests were discarded.

The counting of the colonies on the plates was done with the aid of a hand lens. The term "bacteria" used throughout this paper includes yeasts and actinomycetes, since no attempt was made to separate these from the bacteria proper. Molds were noted and recorded separately, but are not given here. In general they were abundant and at times more numerous than the bacteria.

DESCRIPTION OF AEROSCOPES

The term "aeroscope," as used in this paper, indicates an apparatus used to gather bacteria from the air. It does not include the accessories, such as the aspirator bottle and its connections. In the past there has been some variation in the use of the term among different writers. Usually where the principle of filtration through sand was employed, the apparatus has not been called an "aeroscope," while the term is almost always applied to an apparatus in which a liquid is used as the filtering agent.

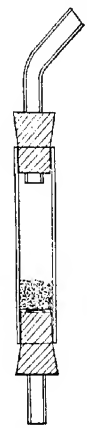

FIG. 1.—Standard aeroscope.

There seems no good reason for this distinction. Likewise there seems to be very little justification for restricting the word to a part of the apparatus, as is done by Rettger (22).

The aeroscope referred to in this paper (fig. 1) as the "standard" is constructed as follows: A 10-mm. layer of sand which has been passed through a 100-mesh sieve and has been retained by a 200-mesh sieve is supported within a cylindrical glass tube 70 mm. in length and 15 mm. in diameter upon a layer of bolting cloth folded over the end of a rubber stopper. Through a perforation in the stopper there passes a tube 6 mm. in diameter and 40 mm. in length. This tube is attached to the aspirator bottle. The upper end of the cylindrical tube is closed by a perforated rubber stopper through which is passed a glass tube 40 mm. in length and 6 mm. in diameter bent at an angle of 45° in order to prevent precipitation of bacteria or dust particles into the aeroscope.

FIG. 2.—Modified standard aeroscope.

In using this aeroscope a measured volume of air is filtered through the tube, the sand shaken out into 10 c. c. of sterile water, and aliquot portions of this suspension plated on nutrient agar.

The "modified" form (fig. 2) of the standard aeroscope (fig. 1) differs from the standard in that the lower rubber-stopper and bolting-cloth supports are eliminated and the small tube is fused into the larger one. The layer of sand is supported by a layer of cotton resting on the shoulder

at the junction of the large and small tubes. The upper stopper is replaced by a cork stopper, a construction that permits the aeroscope to be sterilized by dry heat instead of by steam. After this tube was devised it was discovered that Koning (16) had used one of similar construction, although he used a coarser sand in the filter. In this work the sand used in the standard aeroscope and in the modified standard aeroscope was of such a size that it passed a 100-mesh sieve but was retained by a 160-mesh sieve.

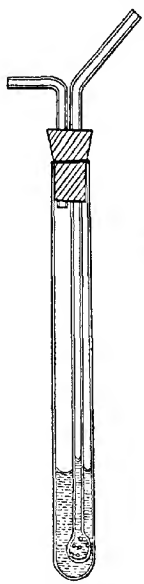

FIG. 3.—Rettger's aeroscope.

Rettger's aeroscope may be described in his own words (22, p. 462–463):

> The entire special apparatus [fig. 3] consists of a glass tube with a small round bulb at one end. The bulb has 8 or 10 small perforations, which serve the purpose of allowing the air to pass through at a rapid rate and yet divide the gas to such an extent that every particle of it is brought into close contact with the filtering fluid. This glass tube or aeroscope is fitted into a small, thick-walled test tube by means of a rubber stopper, which also bears, besides the aeroscope, a short glass tube bent at right angles. The upper end of the aeroscope is bent at an angle of about 45°, in order to prevent bacteria and particles of dust from falling into the open end of the tube, and still permit of the tube being drawn through the stopper without difficulty.

Five c. c. of physiological salt solution are used as the filtering agent. This is plated in aliquot portions, after drawing a measured quantity of air through the aeroscope.

EXPERIMENTAL DATA

Comparison of Various Types of Aeroscopes

The problem of demonstrating which of two aeroscopes is the more accurate is attended with certain practical difficulties. It is not practicable to set up two aeroscopes side by side and determine which is the more reliable, since the bacterial quality of the air is not uniform enough to make it possible to secure strictly comparable results. For this reason some authors have frankly given up this procedure and have resorted to determinations of the filtering efficiency of each filter independently of the other. In these efficiency tests the aeroscopes are set up in tandem—that is, end to end—so that the air passes through one filter and then through the other. The percentage efficiency of the aeroscope is determined by calculating the percentage of the total number of bacteria that appear in the first aeroscope. Percentages obtained by two different aeroscopes in this way ought not to be compared unless they have been calculated from numbers of approximately equal size. The fairest way of making these comparisons is to carry on the two kinds of tests simultaneously. This was not realized in this work until contradictory results had been obtained by the use of the tests separately.

STUDY OF SAND FILTERS IN TANDEM

A series of tests was made, using a sand-filtration method similar in all respects to the standard method except that the rubber stopper with the small tube was omitted from the top of the aeroscope. Two aeroscopes were set up in tandem—i. e., connected end to end by means of a glass tube and two rubber stoppers. In these tests, 5 liters of air were drawn through the filters. No attempt was made to seal the connections leading to the aspirator bottle, but they were made carefully in an attempt to avoid leakage. The plating was done as described on page 346, and in such a way that each plate represented 1 liter of air. In this series of 28 tests the average number of colonies developing per liter was 59 in the first tube (75.9 per cent) and 18.7 in the second tube (24.1 per cent). If three cases, where leakage about the connections between the two aeroscopes evidently occurred, are left out of the calculation, 25 tests gave an average of 50 colonies developing per liter for the first tube (91.4 per cent) and 4.9 per liter for the second tube (8.6 per cent). Later results indicated that slight leakage about the apparently tight stoppers may have influenced even the latter results.

One of the difficulties constantly encountered in air-filtration work is leakage. When this takes place at some point between the filter and the aspirator, the result is that some of the air does not pass through the filter, and therefore the results are low. When, as is probable in the three cases mentioned above, the leakage takes place between the two aeroscopes set up in tandem, the result is that the air goes through only the second filter, making the percentage efficiency of the filter appear lower than it really is.

Another possible condition may have had a marked influence on these results. At times, when sand-filter tubes are sterilized in the autoclave, the sand sticks together to such an extent that cracks appear in it. Such cracked filters are obviously not capable of catching all of the bacteria because of the open pores or lanes through which they may pass. In all of the tests of this series, however, as in all later series, such obviously leaky filters were never used, so that the results given all favor the standard aeroscope to that extent.

TESTS OF RETTGER AEROSCOPES IN TANDEM

A series of 33 tests was run with the Rettger aeroscopes in tandem in order to determine their filtering efficiency. In this work again the difficulty in securing absolutely tight joints was not properly appreciated at first, and rubber connections were used between the two aeroscopes. After sterilization in steam these rubber connections always had a tendency to loosen, and while they were always examined to make sure that they were tight, yet the results indicate that leakage did occur in three cases at least. Seven liters of stable air were drawn through each aeroscope. The aeroscopes contained 5 c. c. of physiological salt solution. One c. c. of the resulting suspension was used in making the plates, agar being

used as the nutrient medium. The average plate count for the first tube in the 33 tests was 18.8, and 2.6 for the second tube (88 per cent efficiency). When the three evident cases of leakage are left out of consideration, the averages become 19.8 colonies per plate for the first tube and 1.5 colonies per plate for the second tube (93 per cent efficiency). In spite of the possible leakage and the fact that the results are all so low that the experimental error due to possible contaminations in plating is high, the results show the Rettger aeroscopes to be fairly efficient.

COMPARATIVE TESTS WITH SAND AND RETTGER AEROSCOPES

Comparative tests were then made between the sand aeroscopes and the Rettger aeroscopes constructed as described (p. 348), but set up singly instead of in tandem. The openings of the two aeroscopes were so placed that they were less than 1 inch apart, and 7 liters of air were drawn through each at the same rate by two aspirator bottles of the same construction. Thus, the filters had an opportunity to obtain air of the same bacterial quality. In order to make the agar plates comparable, 5 c. c. of water were used to wash the sand from the sand filter. One c. c. of each suspension was used in making the plates. The results of these analyses are given in Table I. The averages of the 25 tests are 68 colonies per plate for the sand aeroscope and 30 per plate for the Rettger aeroscope. When calculated on the per liter basis, these averages become 48.6 for the sand aeroscope and 21.5 for the Rettger aeroscope. The same number of colonies per plate was obtained from each aeroscope in six tests. There was 1 excess in favor of the Rettger aeroscope, while 18 excesses were in favor of the sand aeroscope. Six tests were omitted from this table because of bad check plates, but the inclusion of these would not have affected the general results.

TABLE I.—*Comparative counts obtained with the Rettger and an early form of the standard aeroscope*

No. of test.	Agar plate counts.		Counts per liter.		No. of test.	Agar plate counts.		Counts per liter.	
	Rettger.	Standard.	Rettger.	Standard.		Rettger.	Standard.	Rettger.	Standard.
1	14	10	10.0	7.1	15	48	216	34.3	154.3
2	4	4	2.8	2.8	16	11	32	7.8	22.8
3	4	4	2.8	2.8	17	91	21	65.0	15.0
4	5	14	3.5	10.0	18	37	123	26.4	87.8
5	2	8	1.4	5.7	19	53	96	36.4	66.5
6	24	66	17.1	47.1	20	19	67	13.5	47.9
7	14	33	10.0	23.5	21	35	58	25.0	41.4
8	3	4	2.1	2.8	22	23	32	16.4	22.8
9	0	8	0	5.7	23	15	118	10.7	84.3
10	3	6	2.1	4.3	24	24	226	17.1	161.4
11	31	30	22.1	21.4	25	26	91	18.6	65.0
12	23	118	16.4	84.3					
13	9	17	6.4	12.1	Average.	30	68	21.5	48.6
14	237	302	169.3	215.7					

Thus far the results were contradictory, since the tests where the aeroscopes were placed in tandem had shown a greater efficiency for the Rettger filters than for the sand filters, while in the duplicate tests of the two types of aeroscopes the sand filters gave better results. Moreover, there were several unsatisfactory features of the tests. Possible leakage had not been perfectly eliminated, the number of bacteria present in the air was so low that slight errors due to contaminations were greatly magnified, and the sterilization of the sand filters with steam produced unsatisfactory results. The latter caused the sand to cake more or less, as already pointed out, and soon injured the rubber stoppers and connections to such an extent that leakage was always a possibility.

At this point a modification of the standard method was tested. A cotton layer sufficiently firm to support the column of sand was inserted just above the lower rubber stopper. The lower rubber stopper was then removed until the aeroscope proper had been sterilized with dry heat. After sterilization the rubber stopper was carefully inserted. Two series of tests of this modified form of aeroscope were made, one of 43 and the other of 38 trials, but the results were so contradictory and inconclusive that they are not given in detail. At this time the modification described and illustrated (fig. 2) was devised.

COMPARISON OF THE EFFICIENCY OF THE MODIFIED STANDARD AND THE STANDARD AEROSCOPE

In comparing the efficiency of the aeroscopes of the standard type with those of the modified type, the aeroscopes were set up in tandem and the tandem couples of each type of filter were used for duplicate analyses. The openings of the aeroscopes were always placed side by side, and similar aspirators were used so that the rate of filtration could be kept the same. The samples of air were all taken in the stable when the cows were in their stalls and during such operations as feeding or milking. This was done because previous experience had shown that the number of bacteria in the air of the stable when it was empty or when everything was quiet was so low that very few colonies appeared on the plates. In every case the sand was shaken out into 10 c. c. of sterile physiological salt solution. One c. c. of the resulting bacterial suspension was added to each plate. Plating was done in duplicate in the first series given in Table II and in triplicate in the second series.

TABLE II.—*Comparative counts obtained with modified standard and standard aeroscope set up in tandem*

CONNECTIONS NOT SEALED. DUPLICATE PLATING

Test No.	Modified standard aeroscope.			Standard aeroscope.		
	Plate count in first tube.	Plate count in second tube.	Count per liter.	Plate count in first tube.	Plate count in second tube.	Count per liter.
1	536	1	335	.....	6	.....
2	175	68	109	.....	31	.....
3	234	0	146	131	13	82
4	32	1	20	10	0	6
5	54	0	34	118	1	74
6	289	1	181	249	4	156
7	165	4	103	3	6	2
8	81	4	51	26	7	16
9	258	42	161	138	189	86
10	288	46	180	277	33	173
11	174	4	109	122	19	76
12	581	7	363	506	12	316
13	443	5	277	459	99	287
14	64	2	40	53	12	33
15	51	7	32	165	22	103
16	140	6	88	191	15	119
Average	217	12	139	175	29	109

CONNECTIONS SEALED WITH PARAFFIN. TRIPLICATE PLATING

1	185	0.5	116	116	15	73
2	511	1.5	319	582	17	364
3	382	11.0	239	378	80	236
4	500	2.0	313	485	18	303
5	382	.5	239	394	25	246
6	128	6.0	80	164	22	103
7	179	6.5	112	137	17	86
8	185	0	116	120	12	75
9	185	2.0	116	222	75	139
10	391	4.5	244	437	24	273
11	436	3.0	273	328	39	205
12	364	1.0	228	273	32	171
13	854	4.7	534	1,031	90	644
14	813	1.3	508	803	77	502
15	1,050	9.0	656	943	131	589
16	1,281	1.3	801	857	152	536
17	248	1.0	155	237	7	148
18	225	1.7	141	273	7	171
19	338	4.3	211	359	52	224
20	273	3.0	171	287	139	179
21	204	1.7	128	248	8	155
22	136	2.0	85	195	7	122
23	322	3.0	201	391	1	244
24	224	1.3	140	143	9	89
25	217	1.0	136	218	3	136
Average	400	3.0	250	384	42	240

In the first series of tests reported in Table II the joints between the two filter tubes were not sealed to prevent leakage, but were carefully examined to discover possible leakage. As the results show (see tests 2, 9, and 10), some leakage probably did occur through the cork stoppers which were used to connect the tubes. For this reason, in the second series of tests all joints which could possibly leak were sealed with paraffin after the apparatus had been sterilized.

The first series of tests (Table II) gave an average of 217 colonies per plate for the first aeroscopes of the tandem pairs in the modified form of aeroscope, with 12 per plate (5.7 per cent) for the second aeroscopes. In comparison with this, the first tubes of the selected (p. 349) standard aeroscopes gave an average of 175 colonies per plate, with 29 colonies per plate (13.2 per cent) for the second aeroscopes of the tandem pairs.

Twenty-five comparative tests in the second series (Table II) under the rigid conditions just described gave an average of 400 colonies per plate for the first aeroscope of the modified form and 3 per plate (0.75 per cent) for the second. The selected standard aeroscopes gave an average of 384 colonies per plate for the first aeroscopes and 42 per plate (about 10 per cent) for the second ones. Thus, while the standard aeroscopes allowed an appreciable number of bacteria to pass through into the second tube, the modified form probably did not allow any bacteria to pass through into the second tube, the small numbers of colonies occurring on the plates being scarcely more than the numbers that appeared on the check plates.

The second series (Table II) shows conclusively that the standard aeroscopes allow bacteria to pass through the sand layer, since all other leakage was cut off by the paraffin seal. It is probable that a more careful selection of those standard aeroscopes in which the layer of sand was least affected by the steam sterilization would have resulted in a higher average efficiency than was found in these tests. On the other hand, if no selection had been made, the results would undoubtedly have shown a much lower average efficiency. The mere fact that such a selection is necessary in order to secure reliable results is of itself a serious weakness in the standard procedure.

The idea that the low numbers on the plates of the second aeroscope in the train of the modified standard aeroscope really represent contaminations is further supported by the fact that an examination was made of the cotton plugs used to support the sand filter in the modified aeroscope. In 25 of the tests, after the sand in the first tube had been poured out, the upper end of the tube that had been in contact with this sand was carefully flamed and the cotton plug blown out into a test tube containing 10 c. c. of sterile water. After a thorough shaking, this bacterial suspension was plated in duplicate in 1 c. c. quantities. In the entire series the average number of colonies that developed per cotton plug was only 50 (5.0 colonies per plate), the highest number

being 132 (13.2 colonies per plate) and the lowest 0. Undoubtedly a large part, if not all, of the bacteria present were really in the sand or clinging to the walls of the aeroscope and were scraped out of the tube with the cotton plug. Even if they were all present in the cotton, they represent less than 1 per cent of the total number of bacteria caught.

All of the analyses thus far described were made by the writer. Since it was desirable to know whether another worker would get equally good results with this modified form of aeroscope and also whether the sand filter was equally efficient when a more rapid current of air was passed through it, Mr. Kulp, who had never before had experience with bacterial air analyses, undertook a new series of analyses. In this series of 22 tests, the air was aspirated at the rate of 1 liter per minute or even faster in some cases (7 liters in five minutes), while all of the previous analyses had been made with an aspiration rate of 1 liter in two minutes. Connections were sealed with paraffin, as before. The material was plated on both agar and lactose agar, but only the results of the former will be discussed here. The samples were plated in triplicate, as in the preceding series. The results of this series of tests are given in Table III.

TABLE III.—*Efficiency tests of the modified standard aeroscope*

[Aeroscopes set up in tandem. Aspiration more rapid than in previous tests]

Test No.	Plate count in first tube.	Plate count in second tube.	Count per liter.	Test No.	Plate count in first tube.	Plate count in second tube.	Count per liter.
1	58	6	83	14	56	7	80
2	48	4	69	15	93	11	133
3	29	1	41	16	75	7	107
4	45	13	64	17	60	2	86
5	28	3	40	18	43	2	61
6	138	1	197	19	15	0	21
7	205	2	293	20	66	0	94
8	282	1	403	21	43	1	61
9	94	1	134	22	30	1	43
10	74	2	106	23	43	1	61
11	38	2	54				
12	66	0	94	Average	76	3	105
13	55	0	79				

From this table it will be seen that the first tube of the modified form of aeroscope gave an average of 76 colonies per plate (96.3 per cent), while the second tube gave 3 colonies per plate (3.7 per cent). The cotton plugs were plated as before, resulting in the development of 36 colonies per plug (3.6 colonies per plate). Since the number of colonies that appeared on the plates made from the second tube was the same as in the previous series (Table II, second series), it was felt that the lower percentage of efficiency in this case was largely the result of the fact that fewer bacteria were present per liter of air.

COMPARISON OF THE EFFICIENCY OF THE MODIFIED STANDARD AND THE RETTGER AEROSCOPE

Since the previous work with the Rettger aeroscope had not given conclusive results, another series of tests was carried out. As before, two large aspirator bottles were used and 8 liters of air were drawn through each filter at the rate of ½ liter per minute. The aeroscopes were set up in tandem and all joints carefully sealed with paraffin. In this case the suspensions were plated upon two different media, but only those results secured by the use of plain nutrient agar will be discussed at this place, the other results being referred to later. (See p. 363.) Since the results given as plate counts from the two aeroscopes are not comparable because the Rettger suspensions consisted of 5 c. c. and the sand suspensions of 10 c. c. of physiological salt solution, they have also been computed per liter of air. The latter figures are given in the fourth and seventh columns of Table IV. In this series of comparisons the Rettger suspensions were plated in duplicate and the sand suspensions in triplicate.

TABLE IV.—*Comparative counts obtained with modified standard and Rettger aeroscopes*

Test No.	Modified standard aeroscope.			Rettger aeroscope.		
	Plate count of first tube.	Plate count of second tube.	Count per liter.	Plate count of first tube.	Plate count of second tube.	Count per liter.
1	171	9.3	.214	35	5.0	22
2	88	2.3	110	47	1.5	29
3	122	4.0	153	61	2.5	38
4	125	2.6	156	59	10.5	37
5	73	1.3	91	69	9.0	43
6	117	2.0	146	62	19.0	39
7	91	0	114	27	10.5	17
8	73	2.3	91	43	1.0	27
9	46	1.6	58	32	7.0	20
10	48	1.3	60	22	0	14
11	45	1.0	56	10	1.0	6
12	79	1.0	99	27	3.0	17
13	115	1.6	144	27	1.0	17
14	132	1.6	165	63	1.5	39
15	130	2.6	163	15	15.0	9
Average	97	2.3	121	40	5.8	25
16	51	2.3	.64	69	5.5	43
17	47	.6	59	131	.5	82
18	73	0	91			
19	79	1.0	99	87	0	54
20	68	1.6	85	74	2.0	46
21	178	2.3	223	31	.5	19
22	81	1.6	101	44	.5	28
23	72	.6	90	15	2.0	9
24	47	1.0	59	13	3.0	8
Average	77	1.0	97	58	1.8	36

A break has been made in Table IV because of a slight change which was made in the method of plating the bacterial suspensions from the Rettger tubes. In the first 15 analyses the aeroscope arm through which the air enters was washed out once, just before the bacterial suspension was used for plating, by drawing the suspension up into this arm by suction and then releasing it. This was done because it was realized that there was danger that some bacteria would be lost by clinging to the moist inner surface of this tube. As the counts were made, it became evident that the number of colonies appearing per liter from the Rettger aeroscope was less than the number appearing per liter from the modified sand aeroscope. For this reason, beginning with test No. 16 the rinsing of the entrance arm to the Rettger aeroscope was done more thoroughly (several times). By this means the number of colonies appearing on the Rettger plates was increased, showing that the surmised effect of this moist tube was probably true. This effect of the long entrance tube in reducing the number of bacteria in the water filter is also realized by Rettger, for he states (22, p. 467) that one may expect as high as a 15 per cent error in this way. For this reason he recommends drawing the tube out of the aeroscope (after the steam sterilization and before use) and flaming it in order to make sure that the tube will be dry when used. This was not done in the present series of tests, because it was felt that this manipulation after sterilization introduced too great a chance of accidental contaminations.

The results secured before and after the change of procedure noted were as follows: When the entrance tube was rinsed but once, the Rettger aeroscope gave an average of 25 and the modified standard aeroscope gave 121 colonies per liter. After the rinsing was done more thoroughly, there was still a large discrepancy in results, the Rettger filter giving an average of 36 and the modified sand filter an average of 97 per liter.

In the first 15 tests the average counts obtained from the second tube of each tandem pair was 2.3 colonies per plate for the modified standard aeroscope and 5.8 per plate in the case of the Rettger aeroscope. On the basis of these numbers, the percentage efficiencies calculated for the two aeroscopes are 97.7 and 87.3, respectively. The low percentage efficiency obtained with the Rettger aeroscope is somewhat misleading, owing to the fact that in this case the total numbers obtained by both aeroscopes of the tandem pair are low. In the last nine tests the percentage efficiencies were 98.8 for the modified standard aeroscope and 97.1 for the Rettger aeroscope.

COMPARISON OF RETTGER AEROSCOPE WITH THREE TYPES OF THE MODIFIED STANDARD AEROSCOPE

In order to ascertain, if possible, the reason why the Rettger aeroscopes had given such decidedly lower results in the previous series of analyses than the sand filters, another series of analyses was made. From the data already secured it did not seem probable that the lower figures could be explained entirely by loss due to the clinging of bacteria to the entrance tube. For that reason a series of tests was planned in order to discover whether the difference in the size of the entrance tubes had any effect on the number of bacteria caught in the filters. Four types of aeroscopes were used: (1) The Rettger aeroscope; (2) the modified standard aeroscope used in the preceding experiments; (3) an aeroscope of the same type as No. 2 except that the entrance tube was of the same diameter and length as the one used in the Rettger aeroscope; and (4) an aeroscope of the same type as the second except that the top was left wide open when the air was being drawn through it, precipitation of bacteria and dust being prevented by means of a screen placed above it.

Ten liters of air were drawn through each aeroscope at the rate of ½ liter per minute. Five c. c. of sterile water was used in the Rettger aeroscope as the filtering agent and the same quantity of sterile water was used in making the bacterial suspensions of the sand in the other aeroscopes. Plating was done in triplicate in the usual manner, the entrance tubes to the Rettger aeroscopes being more thoroughly rinsed (about 12 times) than had previously been done. The results of 21 such comparative tests computed on the basis of per liter counts are given in Table V. The averages of the tests showed that the plates made from the Rettger aeroscopes (column 1) developed 90 colonies per liter, those from the modified standard aeroscopes (column 2) developed 90 per liter, those from the modified aeroscopes with the small opening (column 3) developed 97, while those from the modified aeroscope protected by a shield (column 4) developed 80 per liter. Individual results varied greatly, sometimes one aeroscope and sometimes another giving the highest numbers. From this series of analyses it seems evident that the size of the opening has little influence upon the number of bacteria caught by the filters, and it also shows that under some conditions the Rettger aeroscope may catch as many bacteria as the sand filters. Just why the Rettger aeroscope should have proved more efficient in the series of tests given in Table V than in the series given in Table IV is not evident from the analyses given, unless it was due to the more thorough rinsing of the inlet tube.

TABLE V.—*Comparative counts obtained with four different types of aeroscopes*

[Bacterial counts given per liter of air]

Test No.	Rettger aeroscope.	Modified standard aeroscope.	Modified standard aeroscope.[a]	Modified standard aeroscope.[b]
1	23	20	19	.....
2	67	34	33	.....
3	26	99	23	.....
4	14	40	12	.....
5	14	19	11	.....
6	98	265	143	.....
7	73	73	305	110
8	52	248	48	129
9	38	75	83	155
10	60	120	107	75
11	171	115	39	34
12	30	31	15	25
13	39	40	91	33
14	73	58	101	36
15	27	121	81	111
16	211	95	192	94
17	116	77	83	87
18	77	79	63	72
19	80	229	291	189
20	133	44	124	126
21	31	24	24	26
22	36	28	48	46
23	65	27	42	32
24	38	14	12	33
25	156	178	188	95
26	41	123	53	59
27	343	94	57	98
Average for 21 tests	90	90	97	80
Average for 27 tests	79	88	85	.....

[a] The ordinary inlet tube of this form of aeroscope (see fig. 2) was replaced by an inlet tube of the diameter and length of the inlet tube on the Rettger aeroscope.

[b] No cork or inlet tube was placed in the upper end of these aeroscopes. Precipitation of dust was prevented by means of a shield placed above the opening.

EFFECT OF USE OF LIQUID FILTERS ON COUNT

Since one of the chief differences between the Rettger type of aeroscope and the modified standard aeroscope is in the filter and since there are several possible ways in which the use of a liquid filter might tend to lower the count, further investigations were made. As both distilled water and physiological salt solution had been used in making the tests with the Rettger aeroscopes, series of analyses were made with both of these in which the bacterial suspensions were plated at once and again three hours later. The actual time during which these substances might exert a deleterious influence upon the bacteria was about 30 minutes longer in the case of the Rettger aeroscope than the times given, as it was impossible to make the first plating until about that interval of time had elapsed after first starting the air bubbling through the liquid.

While these tests were being made with the Rettger aeroscope, duplicate tests were run with the modified standard aeroscopes. The bacterial suspensions were plated in this case immediately after being made and

again after an interval of three hours. The detailed results will not be given, since they were somewhat contradictory and bear upon another line of investigation now in progress. In some cases there was a great reduction in numbers of colonies appearing on the plates after the bacterial suspensions had stood for the three hours, in others little or no reduction, and in some cases even an increase. There seemed to be little difference between the action of the distilled water and that of the physiological salt solution.

One interesting result that appeared as the result of these comparative tests was that the Rettger filters in this series of analyses caught nearly though not quite as many bacteria as the sand filters. The exact averages for the 26 tests were 142 colonies per liter for the Rettger aeroscope as compared with 173 for the modified standard aeroscope. Of all analyses given in this paper, 82 give a direct comparison between the Rettger aeroscope and the modified standard aeroscope. Of these 82 comparisons, 49 show larger numbers of bacteria caught by the sand filters, 17 show excesses for the water filter, and 16 give practically the same figures for both. However, if we take only the last 54 of these analyses, omitting the first comparisons, which were not properly carried out in all their details, the showing is more favorable for the Rettger aeroscopes. In these there were 22 excesses for the sand filter, 16 for the liquid filter, and 16 where the results were practically identical.

From these analyses it must be concluded that the Rettger aeroscope is probably very nearly as efficient as the modified standard aeroscope in the hands of an experienced man. There are several things about it, however, that are liable to cause trouble and others that make it less convenient to use. One already mentioned is the possible injurious effect of the liquid used as a filter upon the bacteria before the material can be plated, owing to absorbed gases, unfavorable osmotic action, or other causes making it necessary to complete the plating as quickly as possible after starting the analysis. This one objection makes it inadvisable to use this type of aeroscope for analyses made at a distance from the laboratory. A second difficulty is the need of great care to prevent bacteria from being held on the moist inner surface of the inlet tube. A third is the necessity of steam sterilization, which occasionally loosens the joints about the cork, causing inaccuracies. Since the modified form of aeroscope met all of these difficulties and was cheaper, easier to operate, less likely to break, and more adaptable to field work, it was decided to use this in the investigation to which the work recorded in this paper was preliminary.

COMPARISON OF RESULTS OBTAINED BY DUPLICATE ANALYSES MADE WITH THE SAME TYPE OF FILTER

Inasmuch as the conclusions drawn from data already presented are mainly based upon the averages of from 25 to 30 comparisons, it may be instructive to find out whether such conclusions are justified or whether

they are due to incidental differences in the bacterial quality of the air. Accordingly three series of comparative tests were made, using the same type of filter on each side of the comparison. The first series of 32 such comparisons is recorded in Table VI. In this case two sand filters of the type described on page 351 were used. Six liters of air artificially enriched with dust were drawn through each filter at the same rate. The sand was washed in 5 c. c. of sterile water, and 1 c. c. of the suspension was added to each plate.

TABLE VI.—*Comparative counts obtained with two standard aeroscopes modified to allow dry sterilization*

Test No.	Plate count.		Count per liter of air.	
	Sample.	Duplicate.	Sample.	Duplicate.
1	109	132	91	110
2	358	314	298	262
3	126	357	105	298
4	508	268	423	223
5	323	243	269	203
6	140	89	117	74
7	222	352	185	293
8	104	161	162	134
9	65	73	54	61
10	90	88	75	73
11	126	81	105	68
12	210	186	175	155
13	178	167	148	139
14	318	184	265	153
15	132	157	110	131
16	96	57	80	48
17	710	933	592	778
18	635	602	529	502
19	600	721	500	601
20	862	786	718	655
21	504	212	420	177
22	222	401	185	334
23	384	381	320	318
24	730	886	608	738
25	487	405	406	338
26	370	430	308	358
27	154	194	128	162
28	148	213	123	178
29	20	45	17	38
30	59	25	41	21
31	23	23	19	19
32	93	137	78	114
Average	287	290	239	242

As will be seen from Table VI, 32 tests gave an average of 287 colonies per plate in one sample of air and 290 in the duplicate, or 239 colonies per liter in the sample and 242 in the duplicate. Such close agreement of results, even in series as long as this, are rarely obtained with the technique used. Marked variations occurred in individual cases, however, showing the need of a long series of tests from which to draw conclusions.

DUPLICATE SAMPLING WITH A REVERSIBLE ASPIRATOR

These tests differed from those just given in that the ordinary aspirator bottles were replaced by a reversible aspirator. A Y-connection was placed in the system so that the current of air could be drawn first through one aeroscope and then through the other. Two liters of air were usually drawn through each aeroscope before diverting the current. In this way either 18, 24, or 30 liters of stable air were drawn through each aeroscope, the larger volume being used at the beginning of the series of analyses and the smaller toward the end, when it was discovered that the plates were overcrowded with colonies. Table VII gives the data gathered in this manner. The average numbers of colonies per plate for the two series of 30 tests were 673 and 643. In this comparison the individual results agree a little better than in the previous series, but this is probably partly due to accident and partly to the influence of overcrowded plates. If nine of the comparisons with the most overcrowded plates are left out of the calculation, the averages become 276 for the one set of analyses and 226 for the other. Some individual analyses present wide variations, but the averages show that fairly comparable duplicate results can be secured with this type of aeroscope when used in a series of analyses.

TABLE VII.—*Comparative counts made with two modified standard aeroscopes placed side by side*

Test No.	Number of liters of air.	Plate count.		Count per liter of air.	
		Sample.	Duplicate.	Sample.	Duplicate.
1	24	233	212	49	44
2	24	1,092	1,187	228	247
3	30	485	504	81	84
4	30	434	463	72	77
5	24	341	377	71	79
6	30	204	186	34	31
7	30	12	29	2	5
8	30	379	106	63	18
9	24	1,067	1,281	222	267
10	30	2,121	2,863	354	477
11	24	3,958	1,305	825	272
12	24	1,723	4,445	351	920
13	18	316	329	88	91
14	18	175	190	49	53
15	18	43	118	12	33
16	18	59	28	16	8
17	24	513	561	107	117
18	24	321	102	67	21
19	18	472	198	131	55
20	18	607	1,409	169	391
21	18	868	1,388	241	386
22	18	878	894	244	248
23	18	179	123	50	34
24	18	205	119	57	33
25	18	1,158	674	322	187
26	18	147	283	41	79
27	18	277	146	77	41
28	18	341	404	95	112
29	18	318	115	88	32
30	18	353	154	98	43
Average		643	673	144	150

DUPLICATE SAMPLING WITH RETTGER AEROSCOPES SET UP IN TANDEM

A series of 20 duplicate analyses was made with Rettger aeroscopes, using 18-liter bottles as aspirators and filtering 15 liters of stable air through each aeroscope. Five c. c. of sterile water (used instead of physiological salt solution in order to avoid foaming) was used as the filtering agent and 1 c. c. of the suspension was added to each Petri plate. The aeroscopes were also set up in tandem connected by a continuous glass tube. Table VIII gives the results of these analyses in detail.

TABLE VIII.—*Duplicate counts made with Rettger aeroscopes in tandem couples*

Test No.	Sample.			Duplicate.		
	Plate count of first tube.	Plate count of second tube.	Count per liter.	Plate count of first tube.	Plate count of second tube.	Count per liter.
1	10	0.6	3	24	0.3	8
2	0	.3	0	1	.0	0
3	5	11.0	2	6	6.0	2
4	15	.6	5	12	.3	4
5	31	.0	10	42	.3	14
6	5	.6	2	3	.3	1
7	3	.6	1	4	.3	1
8	12	.6	4	5	.0	2
9	29	9.6	10	17	1.3	6
10	8	3.6	3	8	2.3	3
11	12	.6	4	5	1.0	2
12	71	3.0	24	47	3.0	16
13	41	3.3	14	20	1.0	7
14	171	52.5	57	57	23.6	19
15	35	8.6	12	13	3.6	4
16	52	9.3	17	116	3.0	39
17	166	3.0	55	52	3.0	17
18	51	6.0	17	50	1.0	17
19	132	5.0	44	124	3.3	41
20	108	2.6	36	129	2.3	43
Average	48	6.0	16	39	2.8	12

Many of the results show very few colonies per plate, especially where the analyses were made when the cows were out of the stable and everything was quiet. In this respect these figures are unsatisfactory, since low figures greatly magnify contamination errors. The averages of 20 tests gave 48 colonies per plate for the first sample and 39 for the duplicates. The second tubes of each tandem couple gave average counts of 6 (88.8 per cent efficiency) and 2.8 (93.3 per cent efficiency) colonies per plate, respectively.

These three series of comparative tests show clearly that caution must be exercised in accepting the figures of any single comparison as correct. On the other hand, the average of a series of comparative analyses probably gives reliable results.

COMPARISON OF VARIOUS MEDIA FOR BACTERIAL ANALYSIS OF AIR

In the course of this work several comparative series of tests were made to determine whether or not a more suitable medium than ordinary agar might be found. The media so compared with nutrient agar were as follows: (a) Nutrient gelatin, containing the same ingredients as the agar except for the substitution of 10 per cent of gelatin for 1.5 per cent of agar; (b) a gelatin medium with 20 per cent of gelatin; (c) a gelatin medium made with soil extract (4); (d) lactose agar, containing 1 per cent of lactose in addition to the usual ingredients; and (e) asparaginate agar (4), an agar to which only compounds of known chemical composition have been added.

The problem was not studied exhaustively enough to prove that the agar medium used in this work was the best possible medium for air work, but the data gathered did warrant the conclusion that it was better than any medium compared with it. The last two media mentioned above gave only slightly lower results than the nutrient agar, but the three gelatin media gave decidedly lower results, probably due in part to the lower incubation temperature necessary when using them and in part to liquefaction. The 20 per cent gelatin gave the highest results of the gelatin media, owing to diminished liquefaction of the medium.

COMPARISON BETWEEN TWO METHODS OF MEASURING BACTERIAL PRECIPITATION

In the study of the main problem already mentioned, to which this study of technique was preliminary, it was necessary to determine the number of bacteria precipitated upon a given area in a given time. No standard procedure is given for this by the Committee on Standard Methods of Air Analysis, although it seems evident that there should be such a recognized procedure. This determination is usually made by exposing a Petri plate containing solidified agar or gelatin for a given period of time and counting the colonies that develop on the plates after incubation.

It was felt, however, that this method was entirely inadequate, as it does not give a true measure of the number of bacteria falling on the plate. This comes about because of the possibility that a dust particle may carry more than one bacterium. Ordinarily but one colony develops from a dust particle, so that the number of colonies measures the number of bacteria-laden dust particles falling on the plate rather than the number of bacteria falling on the plate.

The method for determining this bacterial precipitation which was tried was to place 500 c. c. of sterile water in a sterilized pail covered with a metal lid. Check samples were then taken and the lid removed for a given length of time. Samples of the water were then taken after thorough agitation and plated as soon as possible. Later it was discovered that Koning (16, p. 251 et seq.) had used milk as the medium

for catching the precipitated bacteria. Other liquids might be used in the same way.

The satisfactory nature of this technique was demonstrated by the results secured in 34 comparative tests between this technique and the plate-exposure technique. The results are given in detail in Table IX.

TABLE IX.—*Comparison between the exposed-plate and the exposed-pail methods of measuring bacterial precipitation*

Test No.	Number of colonies developed from bacteria precipitated on 1 sq. cm. in a 5-minute interval.		Ratio.	Test No.	Number of colonies developed from bacteria precipitated on 1 sq. cm. in a 5-minute interval.		Ratio.
	Exposed-plate method.	Exposed-pail method.			Exposed-plate method.	Exposed-pail method.	
1	6.0	56	1:9	23	15.7	216	1:13
2	6.4	58	1:9	24	5.9	81	1:13
3	7.8	261	1:32	25	4.9	36	1:7
4	6.1	51	1:9	26	7.4	17	1:2
5	5.2	28	1:9	27	4.3	13	1:3
6	6.0	34	1:6	28	3.4	23	1:7
[a]7	8.7	124	1:14	29	11.1	100	1:9
[a]8	7.8	105	1:13	[a]30	13.1	216	1:16
9	8.4	63	1:8	31	13.2	30	1:2
10	7.2	45	1:6	32	5.5	32	1:6
11	5.4	62	1:11	33	7.1	28	1:4
12	11.6	109	1:9	34	5.9	53	1:9
13	19.9	250	1:12	35	9.2	53	1:6
14	9.3	36	1:4	36	11.0	68	1:6
15	7.0	75	1:11	37	7.7	96	1:13
16	7.7	49	1:7	38	8.3	58	1:7
17	6.3	81	1:13	[a]39	11.1	120	1:10
18	5.5	81	1:15	[a]40	11.6	118	1:10
19	11.0	94	1:9				
20	9.4	184	1:20	Average..	8.1	78.5	1:10
21	10.4	149	1:14				
[a]22	9.2	199	1:21				

[a] These analyses were omitted from the averages because of contaminated check plates.

In tests 1–12 and 23–34 the Petri plates were exposed for five minutes, but this length of time was abandoned because of the overcrowding of the plates. In the other cases, the numbers given were secured by adding the results secured by exposing four different plates consecutively for 1¼ minutes each. In this way the same time of exposure was secured without overcrowding the plates.

The average result secured in these tests was 8 colonies per sq. cm., secured from a 5-minute exposure where this was determined by exposed Petri plates. Where the pail method of exposure was used, the similar figures were 78 colonies per sq. cm. In the most favorable cases the plate-exposure method gave only one half of the numbers secured in the other way, while in the least favorable cases the plate-exposure method gave only 1 colony to 32 colonies which appeared on the plates

made from the pails. Frequently, the colonies that appeared on the plates which had been exposed showed by their very nature that they were of composite origin.

It seems strange that this fundamental weakness of the plate-exposure method has not been properly appreciated by investigators, for it was recognized by Hueppe (14) as long ago as 1891.

This work shows that the figures obtained in all of the air investigations where conclusions are based upon results obtained by the plate-exposure method are not nearly so large as they should have been. The same criticism applies to some of the methods that have been suggested as a means of counting bacteria in air. One of the first to use this faulty principle of regarding bacteria-laden dust particles as equivalent to individual bacteria was Hesse (13). He counted the colonies developing on gelatin after a measured volume of air had been drawn over it in such a way as to catch the dust particles on the gelatin. The idea that the number of colonies developing on the surface of a solid medium after exposure to the air really represents the number of single bacteria deposited seems also to have been held by some of the later investigators, among them being Harrison (12), Russell (23), and Winslow (33).

CONCLUSIONS

It seems reasonable to conclude that the nature of the filters tested had little influence on the results secured in duplicate analyses—that is, those obtained where a sand and a liquid filter were used side by side agreed just as well as those where either two sand filters or two liquid filters were used side by side.

It was found that the particular form of sand-filter aeroscope recommended by the committee on standard methods of bacterial air analysis appointed by the American Public Health Association varied in its filtering efficiency from 50 to 100 per cent, with the average efficiency for two series of tests of 90 and 91.6 per cent. It is believed that the chief cause of error with this form of aeroscope arises from the fact that it is so constructed that it must be sterilized with steam, which causes caking of the sand-filtering layer.

A description is given of a modification of this form of aeroscope, so constructed that it may be sterilized with dry heat. The modified standard aeroscope was found to retain nearly 100 per cent of the bacteria, with little chance of error. It was also found to be cheaper, less breakable, easier to operate, and more adaptable to field work than either the standard sand aeroscope or the aeroscope recommended by Rettger.

The latter can be made to yield excellent results, provided sufficient care is exercised in handling it. Its use, however, is attended with a number of difficulties, among which may be mentioned its tendency to leakage about the rubber stoppers after being sterilized, the foaming of

the liquid during operation, and the tenacity with which the bacteria cling to the inner surface of the moist inlet tube.

The method of determining bacterial precipitation from air by means of exposed Petri plates has been found to be entirely unreliable, as it gives a measure of the number of bacteria-laden dust particles and not a measure of the number of bacteria present. The number of bacteria precipitating upon a given area has been determined by analyzing measured quantities of sterile water which had been exposed to the air for a given length of time in sterile pails. The numbers obtained in this way were from 2 to 32 times higher than those obtained with the plate-exposure method.

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WALLROTHIELLA ARCEUTHOBII

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INTRODUCTION

The fungus *Wallrothiella arceuthobii*[1] has the distinction of causing the only disease of the leafless mistletoes so far described. Comparatively few botanists have seen this interesting fungus either in nature or in museums, and all reference to it has been omitted from works dealing strictly with plant diseases. The fungus was apparently never collected again from the region where it was originally discovered, and no record exists of preserved material from the place of its second discovery. The discovery of the fungus by the writer in the Northwest adds a new interest to its study, especially since it is found to be so abundant as to have some economic significance.

Owing to the fact that the fungus has only been reported twice and from widely separated stations, its literature is very meager. It was originally discovered by Peck,[2] who published a short account of it under the following name and description:

Sphaeria arceuthobii, n. sp.

Perithecia small, densely caespitose, oblong or cylindrical, very obtuse, shining black; asci subclavate, fugacious; spores crowded, globose, colorless, .00016″ in diameter.

Capsules of *Arceuthobium pusillum*. Forestburgh. Sept. (Plate I, figs. 10–14).

It forms little black tufts, crowning the fruit at the tips of the stems and branches. I have not seen it on the staminate plant. I am not fully satisfied that the generic reference is correct, as the perithecia seem to be mouthless. It is interesting to observe the extent to which parasitism prevails. The Arceuthobium is a parasite on the spruce, this fungus is parasitic on the Arceuthobium, and in a few instances a third parasite, a minute white mold, was seen on the perithecia of the fungus.

The second discovery of the fungus was by Wheeler[3] in the Upper Peninsula of Michigan, who reports it as follows:

I found that the mistletoe was also attacked by a fungous parasite, which must have a tendency to check the spread of this pest. Each fruit is attacked at its apex by the fungus *Wallrothiella arceuthobii*, Peck, and, of course, destroyed.

Both Peck and Wheeler published some excellent drawings of the fungus, from which a very good idea of the character of the disease may be obtained.

[1] If the system of classification of Engler and Prantl is employed, the fungus would be referred to Roselinia. (Engler, Adolf, and Prantl, K. A. E. Die natürlichen Pflanzenfamilien. T. 1, Abt. 1, p. 394, 400, 404, fig. 258, A, B. Leipzig, 1897.)

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C—51

HOSTS

The interest arising from the remarkable habit of this fungus of attacking and destroying the immature fruits of *Razoumofskya pusilla* (Peck) Kuntze (*Arceuthobium pusillum* Peck) on the eastern black spruce (*Picea mariana*) has led the writer to search most diligently for it on western species of Razoumofskya. The fungus was not found in any region of the Great Lakes States, although the mistletoe of the spruce was abundant and much material was examined. In the West the search has been more successful. The fungus was first collected on *Razoumofskya douglasii* Englm. (Pl. LV, fig. 1) in the vicinity of Como Lake in the Bitterroot Mountains, Montana. The only tree found bearing infected plants stood in a clump of "left-overs" on a cutting area of the Latchem Lumber Co. The mistletoe is so very abundant in this region and suppresses the Douglas fir (*Pseudotsuga taxifolia*) to such an extent that this tree, according to Supervisor White, of the Bitterroot National Forest, is sometimes omitted altogether from the estimate of the prospective cut. Practically the same conditions prevail throughout the entire Bitterroot and Missoula River Valleys and adjacent regions. It has always been a rule of the writer, in regions of a heavy infection by *R. douglasii*, to look for the small, closely related mistletoe designated by Engelmann "*Razoumofskya douglasii*, var. *abietina*," on *Abies grandis* and *A. lasiocarpa*. Experience has shown that this mistletoe is more likely to occur when its hosts are in the vicinity of the Douglas-fir mistletoe. Whatever conclusions may be drawn from this as to the probable relationship of the mistletoe on *Abies* spp. with the one on *Pseudotsuga* spp., the surmises as to the presence of the former were correct in the present instance.

The mistletoe was discovered once on *A. grandis* by the large spreading or upright brooms. The tree, standing not more than 100 feet from a Douglas fir which supported three immense brooms of *R. douglasii*,[1] was felled and the mistletoe plants carefully examined. A few of the plants, which were pistillate, were found to be infected by *W. arceuthobii* (Pl. LV, fig. 2). On another portion of the same area the fungus was discovered on a few plants (Pl. LV, fig. 3) growing deep within a large broom on *A. lasiocarpa*. Two other mistletoe-infected trees of this species were cut, all that were found in the region, but the parasite was not attacked by the fungus. A search in other sections of the Bitterroot Valley resulted in finding the mistletoe on the grand and alpine firs, but the fungus was not present. At the head of Rattlesnake Creek, a small stream flowing into the Missoula River at Missoula, the fungus was discovered on a small mistletoe (Pl. LV, fig. 4) growing on *Picea engelmanni*. This is the first report of a mistletoe occurring on a spruce in the North-

[1] The brooms caused by this mistletoe are sometimes very large and frequently cause the death of the entire crown above. The infections of no other mistletoe initiate greater and more frequent brooming than do those of *R. douglasii*. For this reason it is one of the most serious parasites of the entire genus.

west. It is apparently the same as that described by Engelmann on spruce from the Sierra Blanca Mountains in northern Arizona under the name "*R. douglasii*, var. *microcarpa*." A single collection of what is apparently the same mistletoe on spruce was made from a recently fallen tree on a cutting area near Laclede, Idaho. The parasite was, however, in a healthy condition. The Douglas-fir mistletoe was found in each of the two last-named regions. The fungus material in all the foregoing cases was very scanty. It is believed, however, that a more protracted search will result in finding the fungus more abundantly on the Douglas-fir mistletoe.

In order to test the ability of the fungus to attack other mistletoes, infected plants of *R. douglasii* were bound in contact with pistillate plants of *R. americana*, the mistletoe of the lodgepole pine (*Pinus murrayana*). To prevent accident, the experiments were protected by binding cheesecloth loosely about the stem supporting the mistletoe, completely inclosing the plants but not interfering with their vital functions. In all, four such experiments were made during the month of October, 1913. Since pollination had already taken place in the early spring, it was inferred that the fruits of the lodgepole-pine mistletoe would mature normally if infection did not occur. In order to have fully mature plants on the same stem for purposes of comparison, small tufts just below the inclosed ones were shielded by a circular piece of thick white cloth tied just above the tuft and hanging down in the form of a loose umbrella. This would not prohibit the circulating spores from coming up under the shield, provided they escaped from the cheesecloth net, but would lessen the chances of inoculation. Furthermore, owing to ascending air currents, spores of fungi usually travel upward or at least not directly downward when starting from an elevated point. Other experiments were initiated on the lodgepole-pine mistletoe by crushing in water a number of mature perithecia and thoroughly spraying the mixture containing spores over a few pistillate plants. To prevent the plants being knocked off during the winter, they were also protected by cheesecloth. These experiments were visited in the latter part of November, 1914. The results were positive. As shown in Plate LV, figure 5, not only had the fruits of the lodgepole-pine mistletoe which were inclosed with the infected plants from the Douglas fir become infected but very thoroughly so. Every fruit bore at its apex the little shiny black tufts of the perithecia of the fungus. One fruit shown to the right in the middle figure of the sprayed plants seemed to have escaped early infection and to have attained nearly a normal size, but, nevertheless, succumbed to the parasite. The tufts of mistletoe just below the infected ones, which were shielded from above, did not become infected and produced normal mature seeds, which were being expelled at the time the experiments were discontinued. The perithecia of the fungus on

R. americana contained mature spores; hence, the life cycle of the fungus in the seed capsule is complete in the fall of the second year coincident with the time required for the ripening of the seeds of the host.

Soon after the conclusion of the experiments, a swamp area in the Kaniksu National Forest, Idaho, scatteringly timbered by lodgepole pine, was visited. The trees were heavily infected with the mistletoes characteristic of this tree. A close examination of the mistletoe plants showed them to be uniformly attacked by the fungus throughout the entire area. So abundant was the fungus that very few of the pistillate plants on any of the trees had escaped attack. This area has been a fruitful source of investigation and a number of important facts have been gathered.

SIGNIFICANCE OF THE FUNGUS TO THE TAXONOMY OF ITS HOSTS

The hosts of *W. arceuthobii*, so far as known at present, are as follows:

Razoumofskya pusilla (Peck) Kuntze on *Picea mariana*.
R. americana (Nutt.) Kuntze on *Pinus contorta*.
R. douglasii (Engelm.) Kuntze on *Pseudotsuga taxifolia*.
R. douglasii, var. *abietina* Engelm., on *Abies grandis* and *A. lasiocarpa*.
R. douglasii, var. *microcarpa* Engelm., on *Picea engelmanni*.

A glance at the foregoing list shows a very interesting association of mistletoes. The form on *A. lasiocarpa* (Pl. LV, fig. 3), as known to the writer, in point of morphology, color, and the time of maturity of pollen and seed, coincides with the form on *A. grandis* (Pl. LV, fig. 2). The mistletoe on *Picea engelmanni* is slightly smaller, often very much so (Pl. LV, fig. 4), but its other characteristics are the same. Comparing these mistletoes with *R. douglasii* and *R. pusilla*, there is at once a marked similarity among all five. They do not vary widely in form and color of the stems. There is some variation in point of distribution of the individual plants on the branch, whether aggregated or appearing singly. Any one mistletoe, however, may exhibit both or either condition. The staminate flowers of all five are a deep rich purple. No other species of the genus possesses this character to such a marked degree. All five bloom at the same time in the same latitude and exposure, and the seeds ripen and are expelled in the same month.

The question naturally arises, What is the true taxonomic position of these mistletoes? Engelmann recognized the close affinities of the small forms on spruce and fir to *R. douglasii* and named them varieties of that species. The isolated and infrequent occurrence of these small mistletoes on spruce and fir in the West should throw some light on their probable relationships. If they are specifically distinct, they should show greater activity in attacking their hosts. As it is, a single tree will bear a few plants (broom formation) and the most diligent search on the same host for miles around will not reveal a second infection. The discovery of *W. arceuthobii* on those forms or species of the same

genus in the West which are most similar to the eastern-spruce mistletoe may have some bearing on the taxonomic position of this group of mistletoes. The occurrence of the fungus on *R. americana*, a very definitely associated and characteristic species with no affinity whatever with the mistletoes of the Pseudotsuga-Abies-Picea group, indicates a cosmopolitan character for the disease. To determine this point, the fungus has been introduced into clumps of the yellow-pine and larch mistletoes. These experiments are now under way.

MORPHOLOGY

Photographs of *W. arceuthobii* have not been published. For this reason detail enlargements from the original negatives of infected and uninfected fruits of *R. americana* are reproduced in Plate LVI, figures 1 and 2. Reproductions of photographs of the fungus (natural size) on all its western hosts, so far as known, are likewise shown (Pl. LV, fig. 1–5). These illustrations indicate very clearly the interesting habitat of the fungus. A study of the enlargements (Pl. LVI, fig. 2) shows the shiny black perithecia densely crowded at the apex of the fruit. Varying numbers, sometimes as many as 40 or more, have been counted springing from the brownish black stroma within the seed capsule. The general shape of the perithecia is that of an oblong cylinder. Usually, however, they are slightly enlarged at the free ends and very abruptly rounded. The hyphæ composing that part of the stroma from which the perithecia take their origin are densely compacted, brown or black, with thick walls. Deeper within the capsule, the brown color is not so conspicuous, although the mycelium is generally brownish. The outer walls of the perithecia are uniformly smooth; very rarely a 4- to 6-celled projection is present. The crowded condition of the perithecia often gives them the appearance of being partially embedded in the stroma. The wall between two perithecia when densely crowded may be very thin and appears to be occasionally ruptured on the escape of the spores from the asci. The asci show considerable variation in shape, owing principally to their crowded condition, but when free are uniformly pear- or club-shaped, with fairly long pedicels. Probably in no other species of the order is the early disappearance of the wall of the ascus so characteristic. Before the spores have reached maturity or at least before they have assumed the normal color of mature spores, the ascus wall disappears. The ascus is probably ruptured at the apex by the pressure of the developing spores within. That a considerable pressure must be exerted against the walls of the ascus is shown by the fact that the spores when free are normally spherical, but within the ascus they are often bluntly angular. They often persist in clumps after their escape from the ascus. The asci vary but very little in size. The measurements (Zeiss filar micrometer with No. 12 compensating ocular

and 8 mm. n. a. 0.65 apochromatic objective) show a close uniformity to those of the type material. The measurements of the asci from fresh material range as follows: 22.3, 22.8, 24, 24.4, 24.8, 25.2μ in length. Evidently considerable shrinkage takes place in stained material, the stained asci measuring 16.5, 16.9, 19.8, 21.9μ in length. The average breadth of the ascus is 3μ. The ascus contains eight unicellular, globose, thick-walled spores. The spores are at first hyaline, but nearing maturity they assume a very conspicuous brown-black color. The color of the mature spores is assumed after their escape from the ascus. The preliminary color changes may, however, take place within the ascus. Prof. Peck, in his original description, states that the spores are hyaline; still he represents, in his illustration (Pl. LV, fig. 14), four mature spores which are black. The change from a hyaline to the pronounced brown or black color was evidently recognized, since "an ascus containing young spores" is represented, after which "four mature spores" that are black are represented. An examination of some of the type material kindly sent the writer by Mr. H. D. House shows the spores in all stages of development and varying from hyaline to black. The dimensions of the spores in the type material are found to agree with the measurements of the spores in the western fungus, which range as follows: Unstained and out of ascus, 3.7, 4.5, 4.9, 5.3, 5.8, 6.2μ; stained, 4.1, 4.5, 4.9, 5.3, 6.2μ. Previous accounts give the diameter of the spores as "about 4μ." The paraphyses are filamentous, short, and very inconspicuous.

All the asci of a single perithecium do not mature their spores together; instead, at the time mature spores are escaping from the perithecium, young asci showing early stages of spore differentiation are discernible. There is consequently a gradual dissemination of the spores, governed to an extent by the humidity of the atmosphere. The opening through which the spores escape is directly at the apex and is formed by the free ends of the thick-walled hypha composing the walls of the perithecium. The cells composing the tips of these hyphæ seem to possess certain hygroscopic properties, as they are observed to bend in or out on the addition or absence of moisture.

BIOLOGY

In what manner the spores of *W. arceuthobii* are conveyed to the pistillate flower of the mistletoe in nature the writer is not in a position to state definitely. Since isolated infections occur promiscously on different branches of the same tree or on different trees in the same locality, it is evident that the wind is the chief factor in spore dissemination. A fact observed among the infected plants on lodgepole pine of the swamp area previously mentioned supports this view. The area lay with its long axis in the direction of the prevailing winds of the Priest River Valley. An examination of the trees bearing infected plants showed that they were more or less in line with each other and extended

in the direction of the most constant winds. On either side of the most heavily infected area the trees did not support infected plants, although the mistletoe was abundant. Furthermore, large compact brooms always bore the greater number of infected plants on the windward side.

The writer has recently determined that insects to a certainty play a rôle in the pollination of these mistletoes. Hymenopterous insects are chiefly in evidence, but those of other orders are also known to promote pollination. During 1914 grasshoppers in great numbers came out of the Hangman Creek Valley near Spokane and fed upon blooming staminate plants of the large mistletoe growing in profusion on yellow pine of the bench lands. These insects seemed to select only the flowers of the staminate plants for food; but, swarming over the pistillate plants, they deposited some of the pollen that adhered to their bodies. It is as easily possible that the spores of the mistletoe fungus are in a minor degree transported in a like manner. Rain dropping from infected to uninfected plants or running down the pendent branches and dropping off at the tips of the mistletoe plants is probably a factor in distributing the disease on any one tree or broom. It so happened at the field station that a number of newly collected infected capsules were left overnight and a portion of the following day on a glass slide under the microscope. An examination of the slide showed that a number of spores had been expelled and lay in a ring about ½ mm. away from the apex of the perithecium. Evidently there is a slight expulsion of the spores under favorable conditions. This came as a surprise, as the stiff ends of the hyphæ forming the perithecial wall seem to open with difficulty. A number of perithecia collected from fallen capsules in the spring still contained numerous spores. The early disappearance of the ascus within the perithecium precludes any expulsion from this source. The force must arise from the continual maturing and crowding of the spores toward the outward end of the perithecium. Under favorable conditions this pressure may become sufficient to force the spores out through the aperture. It has already been indicated that a pressure seems to exist within the perithecium. This force, though weak, may still be sufficient to cause the spores to land on capsules of the same plant that escaped previous infection.

The spores of the fungus are beginning to ripen and to be expelled from the perithecia in the latitude of northern Idaho about the end of November and are capable of germinating immediately. The method of penetration of the germ tube of the spore into the developing fruit of its host has not as yet been observed. Since a considerable period elapses between pollination and the time actual fertilization takes place in the host, it is quite possible that the germination of the fungus spore coincides with the advance of the pollen tube toward the embryo sac. This would enable the germ tube of the spore to travel toward the ovule of its host by a line of least resistance. In early spring, or at the time

actual fertilization of the mistletoe takes place, those tissues destined to become the seed are, in infected plants, observed to be completely filled or destroyed by the mycelium of the fungus. After infection, the young seed capsule never increases much in size and is entirely dominated by the parasite. The diseased capsules usually fall away during late winter and early spring, which allows time for the infection of the pistillate plants. The drain on the vigor of the mistletoe plant, if all the young capsules are infected, is such that it may also succumb and fall. If only one or two capsules of the plant are infected, it will remain intact, maturing the uninfected fruit of the season and fruiting again the following year. Usually, however, the infection of all the fruits of a mistletoe colony or of all the plants of a broom is so complete that few or no seeds mature.

ECOLOGY

All collections so far made of the fungus have not been at an elevation much greater than 3,600 feet, although its hosts may range well up toward the timber line. This indicates a preference for the conditions of the lower levels, where it is not so much exposed to fluctuations of warmth and moisture. The latter factor is probably of greater influence. Until the fungus is found elsewhere it may be said to prefer the North Temperate regions. Forestburg, N. Y., its first known station, is about on a line with the Upper Peninsula of Michigan, the region of its second discovery, and northern Idaho, where it was last found. This is its geographical and climatic range at present. Developing either on exposed or shaded plants, the fungus seems to favor those growing in shaded positions, such as the inner parts of brooms. Absence of direct sunlight may promote development, but, after the capsule becomes infected, direct sunlight can not have much influence on the maturing of the fungus. The germination of the spores would probably be promoted by an absence of direct sunlight. Warm fall rains, such as occur in northern Idaho, are undoubtedly very favorable to the development and spread of the disease, since in this region the fungus has been found most abundant. In damp river bottoms or on the borders of swamp areas the lodgepole-pine mistletoe, which frequently occurs in profusion in such a habitat, is very likely to be attacked by the fungus. Prof. Peck [1] does not record the conditions under which the fungus was growing at Forestburg, N. Y., but presumably it was a region of considerable humidity. The Upper Peninsula of Michigan, where Prof. Wheeler collected the fungus, is a region of numerous swamps and abundant atmospheric moisture. In view of the fact that the fungus is parasitic on the rather succulent capsule of the mistletoe, atmospheric humidity should not greatly interfere with its life functions, except probably in the initial stages of spore germination. The fungus should thrive on the larch

[1] Peck, C. H. Op. cit.

mistletoe, provided it is susceptible to attack, owing to the usually damp condition of the compact moss-covered brooms. It remains to be seen under just what conditions the fungus will propagate itself. To this end it is being introduced into mistletoe regions of all types of exposure.

The ease with which the fungus seems to infect its host leads the writer to believe that it may be of some economic importance in the control of certain species of mistletoe, at least for small areas. For a mistletoe species to propagate itself, it must produce seeds abundantly, in order to insure the infection of the young growing forest. The proportion of mistletoe seeds actually causing infection to the total number produced is very small indeed. Some fall to the ground; some fall on plants not susceptible; most of them fall on parts of the host too old to be penetrated by the young root of the seed. With the exception of a few rare instances, where infections have been known to occur on wound tissue of mature parts of trees, the writer has not yet found either in nature or by actual inoculation a seed taking effect on any part of its host other than the more tender shoots or their equivalents in tenderness of bark and then only when the primary sinker found its way to a leaf scar, leaf scale, or other more vulnerable irregularities of the substratum. Again, the seed must fall in such a position that the protruding root may directly find its way under a leaf scale or be sheltered by the thick bunch of needles at each node of growth or at the base of a leaf or leaf sheath; otherwise it may fail of its purpose. The seed may germinate and expend its stored materials in the production of a primary root of half an inch or more, but before the growing point can penetrate the stem, provided it is in such a position as to be drawn toward it, the young hypocotyl is exhausted. Very few seeds cause an infection when not very favorably located or directly through the smooth epidermis possessing a suberized layer.

With the exception of the small forms mentioned in this paper most of the members of the genus are prolific seed producers. If so few seeds find a vulnerable point on their hosts even with an abundant production of seed, so much less will the chances of infection be if the seed production is lessened. An estimate of the number of seed that should have been produced by the lodgepole-pine mistletoe on a small broom was about 400. Not a single mistletoe seed on this broom had reached maturity. All were attacked by the fungus. The biologic control of organic agents destructive to plant life is in most cases a thing very much in the realm of fancy. It seems, however, that a fungus of the nature of *W. arceuthobii* may be introduced into mistletoe regions possessing certain climatic conditions with the prospect of reducing the seed production of these parasites, and thus reducing the damage caused by the mistletoe.

SUMMARY

Wallrothiella arceuthobii, a fungous parasite on the false mistletoes of conifers, is reported for the first time in the West.

This fungus, first collected by Prof. Peck in New York and again by Prof. Wheeler in the Upper Peninsula of Michigan, was considered a very rare species until it was found to be of common occurrence in parts of Montana and Idaho.

Several new facts pertaining to the morphology and general behavior of the fungus are established.

Its host range has been greatly extended.

The significant fact that the fungus is found in the West on those forms of species of the same genus which are most similar to the eastern black-spruce mistletoe, its host in the East, is thought to have some bearing on the taxonomic position of this particular group of mistletoes.

Its parasitism on the false mistletoes is found to be of great significance in the control of these parasites, which are so destructive to many western conifers.

PLATE LV

Fig. 1.—*Razoumofskya douglasii* on *Pseudotsuga taxifolia*, infected with *Wallrothiella arceuthobii*. Note that two capsules escaped infection. Natural size.

Fig. 2.—*R. douglasii*, var. *abietina*, on *Abies grandis*, infected with *W. arceuthobii*. Natural size.

Fig. 3.—*R. douglasii*, var. *abietina*, on *Abies lasiocarpa*, infected with *W. arceuthobii*. Natural size.

Fig. 4.—*R. douglasii*, var. *microcarpa*, on *Picea engelmanni*, infected with *W. arceuthobii*. Natural size.

Fig. 5.—Left and right figures showing infection of *R. americana* with *W. arceuthobii* by infected plants of *R. douglasii*. The plants at lower part of figures are normal and fully mature. The middle figure shows infection of *R. americana* by spraying upon the plants a mixture containing spores of *W. arceuthobii*. Natural size.

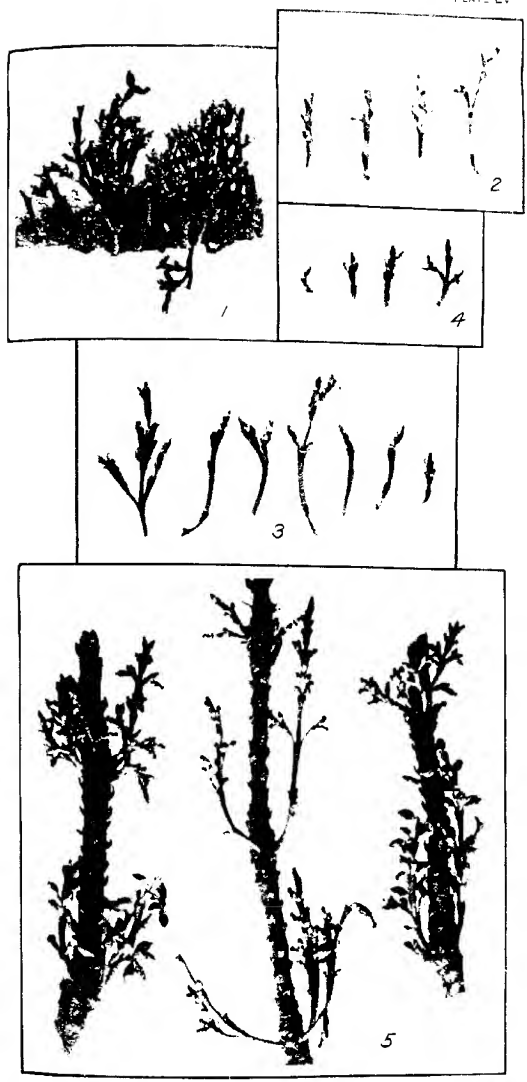
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PLATE LVI

Fig. 1.—Enlargement of the normal fruits of *Razoumofskya americana* shown in Plate LV, figure 5.

Fig. 2.—Enlargement of the diseased fruits of *R. americana* infected with *Wallrothiella arceuthobii* shown in Plate LV, figure 5. Both plants are enlarged to the same scale and show the proportionate size of infected and normal mature fruits.

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